TECHNICAL MANUAL

PowerPlex® ESX 17 System

Instructions for use of Products
DC6720 and DC6721

Revised 6/14
TMD024

PowerPlex® ESX 17 System

Promega

All technical literature is available on the Internet at: www.promega.com/protocols/
Please visit the web site to verify that you are using the most current version of this Technical Manual.
Please contact Promega Technical Services if you have questions on use of this system.
E-mail: genetic@promega.com

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1. Description

STR (short tandem repeat) loci consist of short, repetitive sequence elements 3–7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using the polymerase chain reaction (5–9). Alleles of STR loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using fluorescence detection following electrophoretic separation.

The PowerPlex® ESX 17 System[a–f] is used for human identification applications including forensic analysis, relationship testing and research use. This system allows co-amplification and four-color fluorescent detection of seventeen loci (sixteen STR loci and Amelogenin), including D18S51, D21S11, TH01, D3S1358, Amelogenin, D16S539, D2S1338, D1S1656, D10S1248, FGA, D8S1179, vWA, D22S1045, SE33, D19S433, D12S391 and D2S441.

The PowerPlex® ESX 17 System is designed with the new loci recommended by the European Network of Forensic Science Institutes (ENFSI) and European DNA Profiling Group (EDNAP) loci as mini-STRs (<125bp; D2S441, D10S1248 and D22S1045) or midi-STRs (125–185bp; D1S1656 and D12S391). To complement this design and allow maximal recovery of allelic information from degraded samples, the PowerPlex® ESI 17 Pro System, which amplifies the same seventeen loci present in the PowerPlex® ESX 17 System, is designed with six of the original seven European Standard Set (ESS) loci (D3S1358, D18S51, TH01, vWA, D8S1179 and the more common FGA alleles) along with D16S539 and D19S433 as smaller amplicons (<250bp), while the new ENFSI/EDNAP loci are present as larger amplicons. Therefore, these two STR systems can be used to complement each other when analyzing degraded or challenging samples to maximize recovery of allelic information from as many loci as possible and also allow confirmation of results obtained with the other system.

The PowerPlex® ESX 17 System is compatible with the ABI PRISM® 310, 3100 and 3100-*Avan*t Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers. Amplification and detection instrumentation may vary. You may need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. In-house validation should be performed.

The PowerPlex® ESX 17 System provides all materials necessary to amplify STR regions of human genomic DNA, including hot-start *Taq* DNA polymerase, which is a component of the PowerPlex® ESX 5X Master Mix. This manual contains a protocol for use of the PowerPlex® ESX 17 System with the GeneAmp® PCR system 9700 and 2720 thermal cyclers in addition to protocols to separate amplified products and detect separated material (Figure 1). Protocols to operate the fluorescence-detection instruments should be obtained from the instrument manufacturer.

Information about other Promega fluorescent STR systems is available upon request from Promega or online at: **www.promega.com**

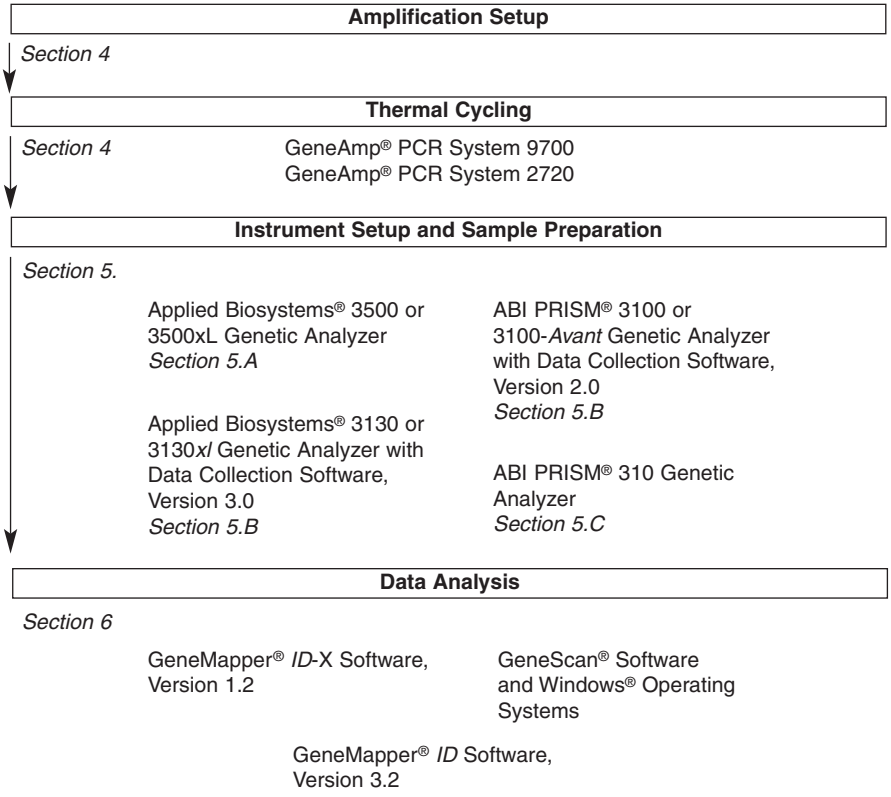
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Figure 1. An overview of the PowerPlex® ESX 17 System protocol.

2. Product Components and Storage Conditions

Product	Size	Cat.#
PowerPlex® ESX 17 System	100 reactions	DC6721

Not For Medical Diagnostic Use. This system contains sufficient reagents for 100 reactions of 25µl each. Includes:

Pre-amplification Components Box

500µl	PowerPlex® ESX 5X Master Mix
250µl	PowerPlex® ESX 17 10X Primer Pair Mix
25µl	2800M Control DNA, 10ng/µl
5 × 1,250µl	Water, Amplification Grade

Post-amplification Components Box

50µl	PowerPlex® ESX 17 Allelic Ladder Mix
300µl	CC5 Internal Lane Standard 500

Product	Size	Cat.#
PowerPlex® ESX 17 System	400 reactions	DC6720

Not For Medical Diagnostic Use. This system contains sufficient reagents for 400 reactions of 25µl each. Includes:

Pre-amplification Components Box

4 × 500µl	PowerPlex® ESX 5X Master Mix
4 × 250µl	PowerPlex® ESX 17 10X Primer Pair Mix
25µl	2800M Control DNA, 10ng/µl
10 × 1,250µl	Water, Amplification Grade

Post-amplification Components Box

4 × 50µl	PowerPlex® ESX 17 Allelic Ladder Mix
4 × 300µl	CC5 Internal Lane Standard 500

The PowerPlex® ESX 17 Allelic Ladder Mix is provided in a separate, sealed bag for shipping. This component should be moved to the post-amplification box after opening. The Water, Amplification Grade, is provided in a separate sealed bag for shipping. This component should be moved to the pre-amplification box after opening.

Storage Conditions: Store all components except the 2800M Control DNA at –30°C to –10°C in a nonfrost-free freezer. Store the 2800M Control DNA at 2–10°C. The Water, Amplification Grade, can be stored at 2–10°C long term. The PowerPlex® ESX 17 10X Primer Pair Mix, PowerPlex® ESX 17 Allelic Ladder Mix and CC5 Internal Lane Standard 500 (CC5 ILS 500) are light-sensitive and must be stored in the dark. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc. For daily use, the PowerPlex® ESX 17 10X Primer Pair Mix and PowerPlex® ESX 5X Master Mix can be stored at 2–10°C for up to 1 month without loss of activity.

Available Separately

The proper panels, bins and stutter files for use with GeneMapper® *ID* and *ID*-X software are available for download at:
www.promega.com/resources/tools/genemapper-id-software-panels-and-bin-sets/.
The PowerTyper™ ESX Macros are available for download at:
www.promega.com/resources/tools/powertyper-macros/

Matrix standards are required for initial setup of the color separation matrix. The matrix standards are provided separately and are available for the ABI PRISM® 310 Genetic Analyzer (PowerPlex® 5-Dye Matrix Standards, 310, Cat.# DG4600) and ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers (PowerPlex® 5-Dye Matrix Standards, 3100/3130, Cat.# DG4700).

3. Before You Begin

3.A. Precautions

The application of PCR-based typing for forensic or paternity casework requires validation studies and quality-control measures that are not contained in this manual (10,11). Guidelines for the validation process are published in the *Internal Validation of STR Systems Reference Manual* (12).

The quality of purified DNA or direct-amplification samples, small changes in buffers, ionic strength, primer concentrations, choice of thermal cycler and thermal cycling conditions can affect PCR success. We suggest strict adherence to recommended procedures for amplification and fluorescence detection. Additional research and validation are required if any modifications to the recommended protocols are made.

PCR-based STR analysis is subject to contamination by very small amounts of human DNA. Extreme care should be taken to avoid cross-contamination when preparing template DNA, handling primer pairs, assembling amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (PowerPlex® ESX 5X Master Mix, PowerPlex® ESX 17 10X Primer Pair Mix, 2800M Control DNA and Water, Amplification Grade) are provided in a separate box and should be stored separately from those used following amplification (PowerPlex® ESX 17 Allelic Ladder Mix and CC5 Internal Lane Standard 500). Always include a negative control reaction (i.e., no template) to detect reagent contamination. We highly recommend the use of gloves and aerosol-resistant pipette tips (e.g., ART® tips, Section 9.E).

Some reagents used in the analysis of STR products are potentially hazardous and should be handled accordingly. Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

3.B. Matrix Standardization or Spectral Calibration

Proper generation of a matrix file is critical to evaluate multicolor systems with the ABI PRISM® 310, 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers. A matrix must be generated for each individual instrument.

The PowerPlex® 5-Dye Matrix Standards, 310 (Cat.# DG4600), is required for matrix standardization for the ABI PRISM® 310 Genetic Analyzer. The PowerPlex® 5-Dye Matrix Standards, 3100/3130 (Cat.# DG4700), cannot be used to generate a matrix on the ABI PRISM® 310 Genetic Analyzer.

The PowerPlex® 5-Dye Matrix Standards, 3100/3130 (Cat.# DG4700), is required for spectral calibration on the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers. The PowerPlex® 5-Dye Matrix Standards, 310, cannot be used to generate a matrix on these instruments.

For protocols and additional information about matrix standardization, see the *PowerPlex® 5-Dye Matrix Standards, 310, Technical Bulletin* #TBD023. For protocols and additional information about spectral calibration, see the *PowerPlex® 5-Dye Matrix Standards, 3100/3130, Technical Bulletin* #TBD024. These manuals are available online at: **www.promega.com/protocols/**

4. Protocols for DNA Amplification Using the PowerPlex® ESX 17 System

The PowerPlex® ESX 17 System is optimized for the GeneAmp® PCR System 9700 thermal cycler. An amplification protocol for the GeneAmp® PCR Systems 2720 thermal cycler also is provided for extracted DNA.

The use of gloves and aerosol-resistant pipette tips is highly recommended to prevent cross-contamination. Keep all pre-amplification and post-amplification reagents in separate rooms. Prepare amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup.

Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section 7.A.

The concentration of 2800M Control DNA was determined by measuring absorbance at 260nm. Quantification of this control DNA by other methods, such as qPCR, may result in a different value. Prepare a fresh DNA dilution for each set of amplifications. Do not store diluted DNA (e.g., 0.25ng/µl or less).

4.A. Amplification of Extracted DNA

Amplification and detection instrumentation may vary. You may need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. The PowerPlex® ESX 17 System is optimized and balanced for 0.5ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different. Testing at Promega shows that 30 cycles work well for 0.5ng of purified DNA templates. Developmental validation of the kit showed routine generation of full profiles using 30 cycles of amplification with lower amounts of DNA template down to 62.5pg. Partial profiles were typically observed for DNA template of 32pg and below (13). In-house optimization and validation should be performed to establish the performance of the kit in your laboratory (12).

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 or 2720 thermal cycler (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plates or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips (see Section 9.E)

Amplification Setup

1. Thaw the PowerPlex® ESX 5X Master Mix, PowerPlex® ESX 17 10X Primer Pair Mix and Water, Amplification Grade, completely.

   Note: Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the primers to be concentrated at the bottom of the tube.

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.

3. Use a clean MicroAmp® plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label appropriately.

4.A. Amplification of Extracted DNA (continued)

4. Add the final volume of each reagent listed in Table 1 to a sterile tube.

Table 1. PCR Amplification Mix for the PowerPlex® ESX 17 System.

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	to a final volume of 25.0µl	×		=	
PowerPlex® ESX 5X Master Mix	5.0µl	×		=	
PowerPlex® ESX 17 10X Primer Pair Mix	2.5µl	×		=	
template DNA (0.5ng)[2,3,4]	up to 17.5µl				
total reaction volume	25µl				

[1]Add Water, Amplification Grade, to the tube first, then add PowerPlex® ESX 5X Master Mix and PowerPlex® ESX 17 10X Primer Pair Mix. The template DNA will be added at Step 6.

[2]Store DNA templates in nuclease-free water or TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA). If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the volume of DNA added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl), available magnesium concentration (due to chelation by EDTA) or other PCR inhibitors, which may be present at low concentrations depending on the source of the template DNA and the extraction procedure used.

[3]Apparent DNA concentrations can differ, depending on the DNA quantification method used (14). The amount of DNA template recommended here is based on DNA concentrations determined by measuring absorbance at 260nm. We strongly recommend that you perform experiments to determine the optimal DNA amount based on your DNA quantification method.

[4]The PowerPlex® ESX 17 System is optimized and balanced for 0.5ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different.

5. Vortex the PCR amplification mix for 5–10 seconds, then pipet PCR amplification mix into each reaction well.

(!) Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.

6. Add the template DNA for each sample to the respective well containing PCR amplification mix.

   Note: The PowerPlex® ESX 17 System is optimized and balanced for 0.5ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different.

7. For the positive amplification control, vortex the tube of 2800M Control DNA, then dilute an aliquot to 0.5ng in the desired template volume. Add 0.5ng of diluted DNA to a reaction well containing PCR amplification mix.

8. For the negative amplification control, pipet Water, Amplification Grade, or TE^{-4} buffer instead of template DNA into a reaction well containing PCR amplification mix.

9. Seal the plate. **Optional:** Briefly centrifuge the plate to bring contents to the bottom and remove any air bubbles.

Thermal Cycling

This section contains a protocol for use of the PowerPlex® ESX 17 System with the GeneAmp® PCR System 9700 and 2720 thermal cyclers.

1. Place the MicroAmp® plate or reaction tubes in the thermal cycler.

2. Select and run the recommended protocol. The preferred protocol for use with the GeneAmp® PCR System 9700 and 2720 thermal cyclers is provided below.

Thermal Cycling Protocol[1]
96°C for 2 minutes, then:
94°C for 30 seconds 59°C for 2 minutes 72°C for 90 seconds for 30 cycles, then:
60°C for 45 minutes
4°C soak

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with 9600 as the ramp speed. The ramp speed is set after the thermal cycling run is started. The Select Method Options screen appears. Select "9600" for the ramp speed, and enter the reaction volume.

3. After completion of the thermal cycling protocol, store amplified samples at –20°C in a light-protected box.

   Note: Long-term storage of amplified samples at 4°C or higher may produce artifacts.

4.B. Direct Amplification of DNA from Storage Card Punches

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate (Applied Biosystems)
- aerosol-resistant pipette tips (see Section 9.E)
- PunchSolution™ Kit (Cat.# DC9271)
- 5X AmpSolution™ Reagent (Cat.# DM1231), also supplied with the PunchSolution™ Kit
- 1.2mm Harris Micro-Punch or equivalent manual punch and cutting mat or automated punch system

This section contains a protocol for direct amplification of DNA from storage card punches using the PowerPlex® ESX 17 System and GeneAmp® PCR System 9700 thermal cycler.

When using the protocol detailed below, add the number of 1.2mm storage card punches indicated below to each 25µl amplification reaction.

Note: You will need to optimize and validate the number of storage card punches per reaction in your laboratory. See the PCR Optimization recommendations at the end of this section.

FTA®-based sample types include:

- Buccal cells collected on FTA® cards with Whatman EasiCollect™ or Fitzco Sampact™ devices (one or two punches per 25µl amplification reaction)
- Buccal cells collected with sterile swabs transferred to FTA® or Indicating FTA® cards (one or two punches per 25µl amplification reaction)
- Liquid blood (from collection or storage Vacutainer® tubes or finger sticks) spotted onto FTA® cards (one punch per 25µl amplification reaction)

NonFTA sample types include:

- Buccal samples on Bode Buccal DNA Collector™ devices (one punch per 25µl amplification reaction)
- Blood and buccal samples on nonFTA card punches (e.g., S&S 903) (one punch per 25µl amplification reaction)

Pretreat nonFTA sample types with the PunchSolution™ Kit (Cat.# DC9271) to lyse nonFTA samples before adding the amplification mix. For more information, see the *PunchSolution™ Kit Technical Manual* #TMD038. Failure to pretreat these samples may result in incomplete profiles.

Use a manual punch tool with a 1.2mm tip to manually create sample disks from a storage card. Place tip near the center of the sample spot, and with a twisting or pressing action, cut a 1.2mm sample disk. Use the plunger to eject the disk into the appropriate well of a reaction plate.

Automated punchers also can be used to create sample disks. Refer to the user's guide for your instrument for assistance with generating 1.2mm disks, technical advice and troubleshooting information.

Note: Static may be problematic when adding a punch to a well. For FTA® card punches, adding PCR amplification mix to the well before adding the punch may help alleviate static problems. For nonFTA card punches, adding PunchSolution™ Reagent to the well before adding the punch during pretreatment may help alleviate static problems.

Amplification Setup

1. Thaw the PowerPlex® ESX 5X Master Mix, PowerPlex® ESX 17 10X Primer Pair Mix and Water, Amplification Grade, completely.

   Note: Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.

2. Vortex the 5X AmpSolution™ Reagent for 10–15 seconds.

   Note: The 5X AmpSolution™Reagent should be thawed completely, mixed by vortexing and stored at 2–10°C. The reagent may be turbid after thawing or storage at 4°C. If this occurs, warm the buffer briefly at 37°C, then vortex until clear. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Storing reagents in the refrigerator door can compromise stability.

3. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.

4. Use a clean MicroAmp® plate for reaction assembly, and label appropriately.

5. Add the final volume of each reagent listed in Table 2 to a sterile tube.

Table 2. PCR Amplification Mix for Direct Amplification of DNA from Storage Card Punches.

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	12.5µl	×		=	
PowerPlex® ESX 5X Master Mix	5.0µl	×		=	
PowerPlex® ESX 17 10X Primer Pair Mix	2.5µl	×		=	
5X AmpSolution™ Reagent	5.0µl	×		=	
total reaction volume	25µl				

[1]Add Water, Amplification Grade, to the tube first, then add PowerPlex® ESX 5X Master Mix, PowerPlex® ESX 17 10X Primer Pair Mix and 5X AmpSolution™ Reagent. For FTA® card punches, the template DNA will be added at Step 6.

4.B. Direct Amplification of DNA from Storage Card Punches (continued)

6. Vortex the PCR amplification mix for 5–10 seconds, then pipet 25µl of PCR amplification mix into each reaction well.

   Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.

7. For FTA® storage cards, add one or two 1.2mm punches from a storage card containing a buccal sample or one 1.2mm punch from a storage card containing whole blood to the appropriate wells of the reaction plate. For nonFTA card punches, add PCR amplification mix to the PunchSolution™ Reagent-treated punches.

   Note: It also is acceptable to add the FTA® card punch first, then add the PCR amplification mix.

8. For the positive amplification control, add 1µl (10ng) of the 2800M Control DNA to a reaction well containing 25µl of PCR amplification mix.

   Notes:

   1. Do not include blank storage card punches in the positive control reactions.
   2. Optimization of the amount of 2800M Control DNA may be required depending on thermal cycling conditions and laboratory preferences. Typically, 10ng of 2800M Control DNA is sufficient to provide a robust profile using the cycle numbers recommended here. A one-cycle reduction in cycle number will require a twofold increase in mass of DNA template to generate similar signal intensity. Similarly, a one-cycle increase in cycle number will require a twofold reduction in the amount of 2800M Control DNA to avoid signal saturation.

9. Reserve a well containing PCR amplification mix as a negative amplification control.

   Note: An additional negative control with a blank punch may be performed to detect contamination from the storage card or punch device.

10. Seal the plate, and briefly centrifuge the plate to bring storage card punches to the bottom of the wells and remove any air bubbles.

    Note: Place the amplification plate in the thermal cycler, and start the thermal cycling program as soon as the PCR amplification mix is added to all wells. Prolonged storage of assembled reactions prior to cycling may result in poor performance (i.e., lower peak heights for large amplicons).

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including the number of storage card punches, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 24 cycles works well for a variety of sample types. Buccal samples may require more amplification cycles than blood samples. Cycle number will need to be optimized in each laboratory for each sample type that is amplified.

1. Place the MicroAmp® plate in the thermal cycler.
2. Select and run the recommended protocol. The preferred protocol for use with the GeneAmp® PCR System 9700 thermal cycler is provided below.

Thermal Cycling Protocol[1]
96°C for 2 minutes, then:
94°C for 30 seconds 59°C for 2 minutes 72°C for 90 seconds for 24 cycles, then:
60°C for 45 minutes
4°C soak

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with 9600 as the ramp speed. The ramp speed is set after the thermal cycling run is started. The Select Method Options screen appears. Select "9600" for the ramp speed, and enter the reaction volume.

3. After completion of the thermal cycling protocol, store amplified samples at –20°C in a light-protected box.

   Note: Long-term storage of amplified samples at 4°C or higher may produce artifacts.

PCR Optimization

Cycle number should be optimized based on the results of an initial experiment to determine the sensitivity with your collection method, sample types, number of punches and instrumentation.

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Depending on your preferred protocol, place one or two 1.2mm storage card punches containing a buccal sample or one 1.2mm punch of a storage card containing whole blood in each well of a reaction plate. Be sure to pretreat nonFTA samples with the PunchSolution™ Kit (Cat.# DC9271).
3. Prepare three identical reaction plates with punches from the same samples.
4. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number.
5. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number for the sample type and number of storage card punches.

4.C. Direct Amplification of DNA from Swabs

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate (Applied Biosystems)
- aerosol-resistant pipette tips (see Section 9.E)
- SwabSolution™ Kit (Cat.# DC8271)
- 5X AmpSolution™ Reagent (Cat.# DM1231), also supplied with the SwabSolution™ Kit

This section contains a protocol for amplifying DNA from swab extracts using the PowerPlex® ESX 17 System and GeneAmp® PCR System 9700 thermal cycler.

Pretreat OmniSwab™ (GE Healthcare) or cotton swabs using the SwabSolution™ Kit (Cat.# DC8271) as described in the *SwabSolution™ Kit Technical Manual* #TMD037 to generate a swab extract.

Amplification Setup

1. Thaw the PowerPlex® ESX 5X Master Mix, PowerPlex® ESX 17 10X Primer Pair Mix and Water, Amplification Grade, completely.

   Note: Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.

2. Vortex the 5X AmpSolution™ Reagent for 10–15 seconds.

   Note: The 5X AmpSolution™Reagent should be thawed completely, mixed by vortexing and stored at 2–10°C. The reagent may be turbid after thawing or storage at 4°C. If this occurs, warm the buffer briefly at 37°C, then vortex until clear. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Storing reagents in the refrigerator door can compromise stability.

3. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.

4. Use a clean MicroAmp® plate for reaction assembly, and label appropriately.

5. Add the final volume of each reagent listed in Table 3 to a sterile tube.

Table 3. PCR Amplification Mix for Direct Amplification of DNA From Swabs.

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	10.5µl	×		=	
PowerPlex® ESX 5X Master Mix	5.0µl	×		=	
PowerPlex® ESX 17 10X Primer Pair Mix	2.5µl	×		=	
5X AmpSolution™ Reagent	5.0µl	×		=	
swab extract	2.0µl				
total reaction volume	**25µl**				

[1]Add Water, Amplification Grade, to the tube first, then add PowerPlex® ESX 5X Master Mix and PowerPlex® ESX 17 10X Primer Pair Mix. The swab extract will be added at Step 6.

6. Vortex the PCR amplification mix for 5–10 seconds, then pipet 23µl of PCR amplification mix into each reaction well.

Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.

7. Pipet 2.0µl of swab extract for each sample into the appropriate well of the reaction plate.

8. For the positive amplification control, vortex the tube of 2800M Control DNA, then dilute to 2.5ng/µl. Add 2µl (5ng) to a reaction well containing 23µl of PCR amplification mix.

   Note: Optimization of the amount of 2800M Control DNA may be required, depending on thermal cycling conditions and laboratory preferences.

9. For the negative amplification control, pipet 2µl of Water, Amplification Grade, or TE^{-4} buffer instead of swab extract into a reaction well containing PCR amplification mix.

   Note: Additional negative controls can be included. Assemble a reaction containing the swab extract prepared from a blank swab, or assemble a reaction where the SwabSolution™ Reagent is processed as a blank without a swab.

10. Seal the plate. **Optional:** Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 26 cycles works well for a variety of sample types. Cycle number will need to be optimized in each laboratory for each sample type that is amplified (see below).

1. Place the MicroAmp® plate in the thermal cycler.
2. Select and run the recommended protocol. The preferred protocol for use with the GeneAmp® PCR System 9700 thermal cycler is provided below.

Thermal Cycling Protocol[1]
96°C for 2 minutes, then:
94°C for 30 seconds 59°C for 2 minutes 72°C for 90 seconds for 26 cycles, then:
60°C for 45 minutes
4°C soak

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with 9600 as the ramp speed. The ramp speed is set after the thermal cycling run is started. The Select Method Options screen appears. Select "9600" for the ramp speed, and enter the reaction volume.

3. After completion of the thermal cycling protocol, store amplified samples at –20°C in a light-protected box.

   Note: Long-term storage of amplified samples at 4°C or higher may produce artifacts.

PCR Optimization

Cycle number should be optimized based on the results of an initial experiment to determine the sensitivity with your collection method, sample types and instrumentation.

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Prepare three identical reaction plates with aliquots of the same swab extracts.

3. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number (25, 26 and 27 cycles).

   Note: This recommendation is for 2µl of swab extract.

4. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number for the sample type.

5. Instrument Setup and Sample Preparation

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3500/3500xL capillary array, 36cm
- 96-well retainer & base set (standard) (Applied Biosystems Cat.# 4410228)
- POP-4® polymer in a pouch for the Applied Biosystems® 3500or 3500xL Genetic Analyzer
- anode buffer container
- cathode buffer container
- MicroAmp® optical 96-well plate (or equivalent) and septa
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

Sample Preparation

1. Thaw the CC5 Internal Lane Standard 500.

   Note: Centrifuge tube briefly to bring contents to the bottom, then vortex for 15 seconds before each use. Do not centrifuge after vortexing, as this may cause the size standard to be concentrated at the bottom of the tube.

2. Prepare a loading cocktail by combining and mixing CC5 Internal Lane Standard 500 and Hi-Di™ formamide as follows:

   [(1.0µl CC5 ILS 500) × (# samples)] + [(10.0µl Hi-Di™ formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of the size standard peaks. Use a volume of CC5 ILS 500 that results in peak heights that are all consistently above the peak amplitude threshold of the orange dye channel determined as part of your internal validation. Keep the volume of formamide at 10.0µl, and adjust the volume added to the wells in Step 4 accordingly.

3. Vortex for 10–15 seconds to mix.

4. Pipet 11µl of formamide/internal lane standard mix into each well.

5. Add 1µl of amplified sample (or 1µl of PowerPlex® ESX 17 Allelic Ladder Mix). Cover wells with appropriate septa.

   Notes:

   1. Instrument detection limits vary; therefore, injection time, injection voltage or the amount of product mixed with loading cocktail may need to be increased or decreased. To modify the injection time or injection voltage in the run module, select "Instrument Protocol" from the Library menu in the data collection software. If peak heights are higher than desired, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles to achieve the desired signal intensity. If the injection time or voltage is reduced, a decreased peak amplitude threshold for the orange channel may be required for proper sizing.
   2. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your internal validation.

6. Centrifuge plate briefly to remove air bubbles from the wells.

7. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

Instrument Preparation

Refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* for the instrument maintenance schedule and instructions to install the capillary array, buffers and polymer pouch and perform a spatial calibration. Samples may be analyzed as described in the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide*.

1. Open the 3500 Data Collection Software. The Dashboard screen will launch (Figure 2). Ensure that the Consumables Information and Maintenance Notifications are acceptable.

   Set the oven temperature to 60°C, then select "Start Pre-Heat" at least 30 minutes prior to the first injection to preheat the oven.

Figure 2. The Dashboard.

2. To create a new Instrument Protocol, navigate to the Library, select "Instrument Protocol", then select "Create". Alternatively, a previously created Instrument Protocol may be used.

   Figure 3 shows the settings used at Promega for the Applied Biosystems® 3500xL Genetic Analyzer for the application type, dye set, capillary length, polymer, run module and appropriate protocol information. The only setting that was changed from the default settings is dye set.

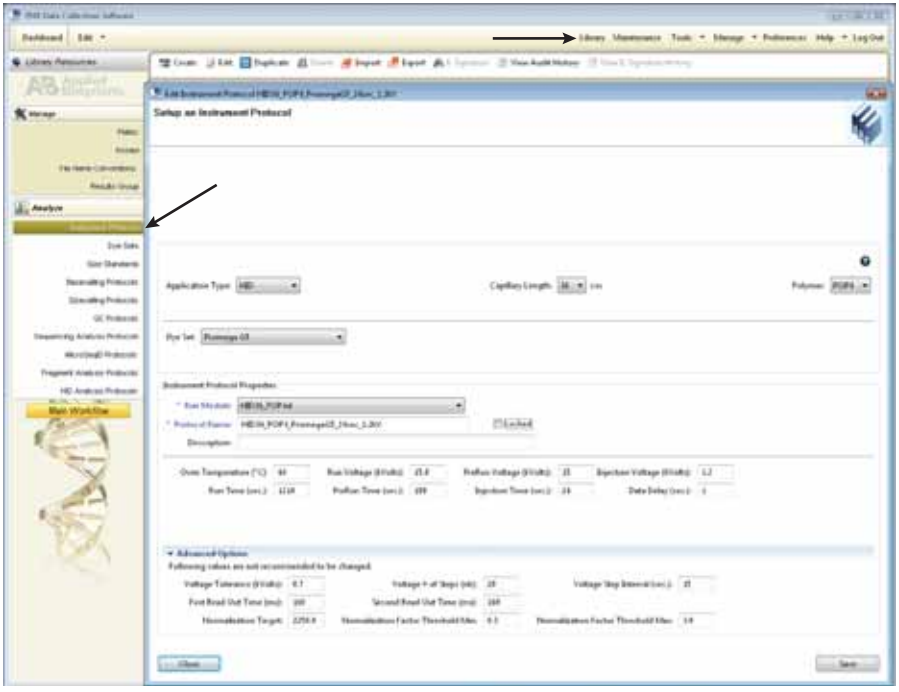

Figure 3. Create New Instrument Protocol window

When creating an Instrument Protocol, be sure to select the same dye set that was used to perform the Promega 5-dye spectral calibration. We recommend using a run time of 1,210 seconds and the default injection conditions.

Run time and other instrument settings should be optimized and validated in your laboratory.

Assign a descriptive protocol name.

Note: For more detailed information refer to the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

3. To create a new Size Standard for the QC protocol, navigate to the Library. Select "Size Standards", then select "Create". Alternatively, a previously created Size Standard may be used.

   Assign the Size Standard the name "ILS500" or another appropriate name. Choose "Orange" as the Dye Color. The fragments in the size standard are 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases. See Figure 4.

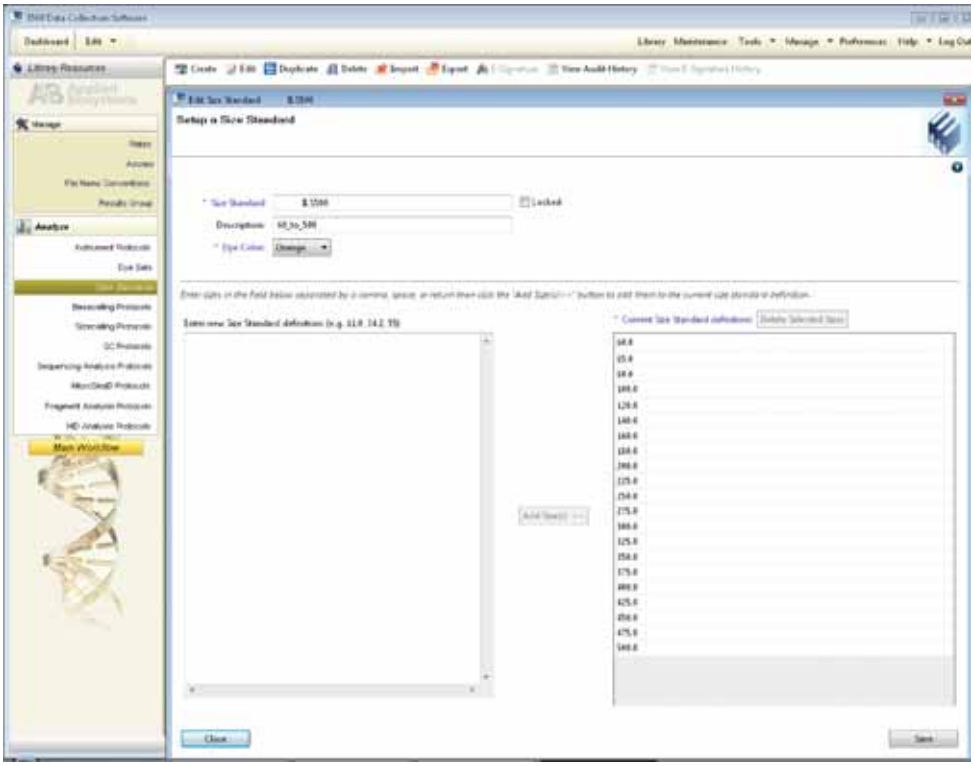

Figure 4. The Create New Size Standard window.

4. To create a new QC Protocol, navigate to the Library. Select "QC Protocols", then select "Create". Alternatively, a previously created QC Protocol may be used.

   Assign a descriptive protocol name. Select the size standard created in Step 3. The settings for the QC protocol should be based on the internally validated conditions for the PowerPlex® ESX 17 System on the Applied Biosystems® 3500 or 3500xL Genetic Analyzer. Figure 5 shows one option for these settings.

   Note: Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes.

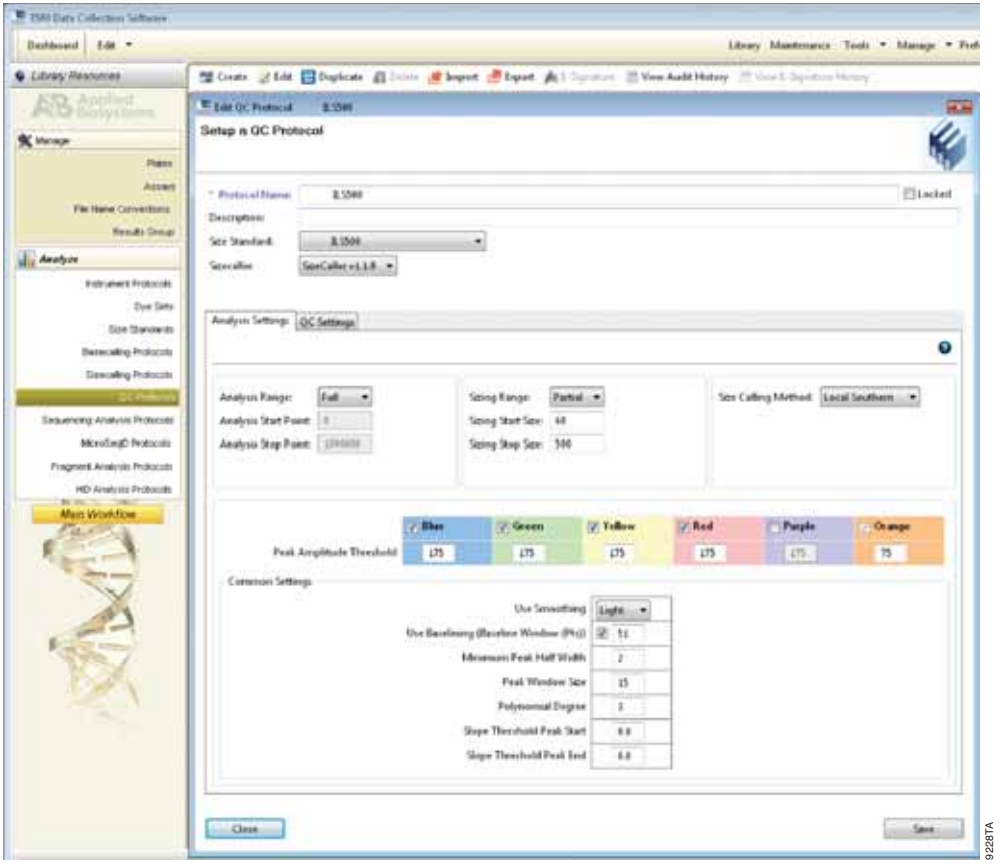

Figure 5. The Create New QC Protocol window.

5. To create a new Assay, navigate to the Library. Select "Assays", then select "Create". Alternatively, a previously created Assay may be used.

   In the Create New Assay window (Figure 6), select the Instrument Protocol created in Step 2 and the QC Protocol created in Step 4. Assign a descriptive assay name. Select the application type "HID". An Assay is required for all named samples on a plate.

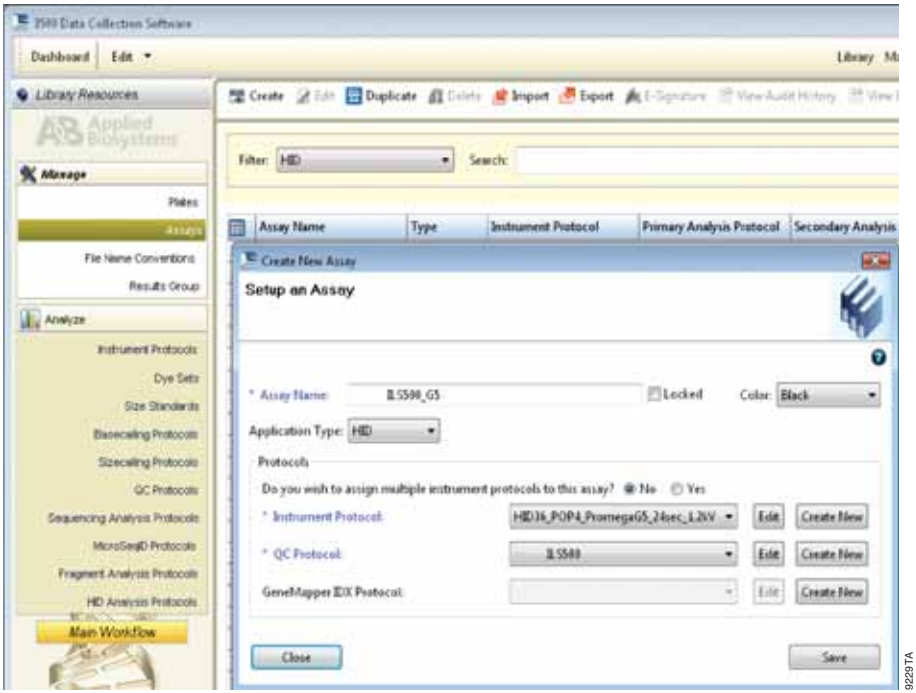

Figure 6. The Create New Assay window.

6. To create a new File Name Convention (Figure 7), navigate to the Library. Select "File Name Conventions", then select "Create". Alternatively, a previously created File Name Convention may be used.

   Select the File Name Attributes according to laboratory practices, and save with a descriptive name.

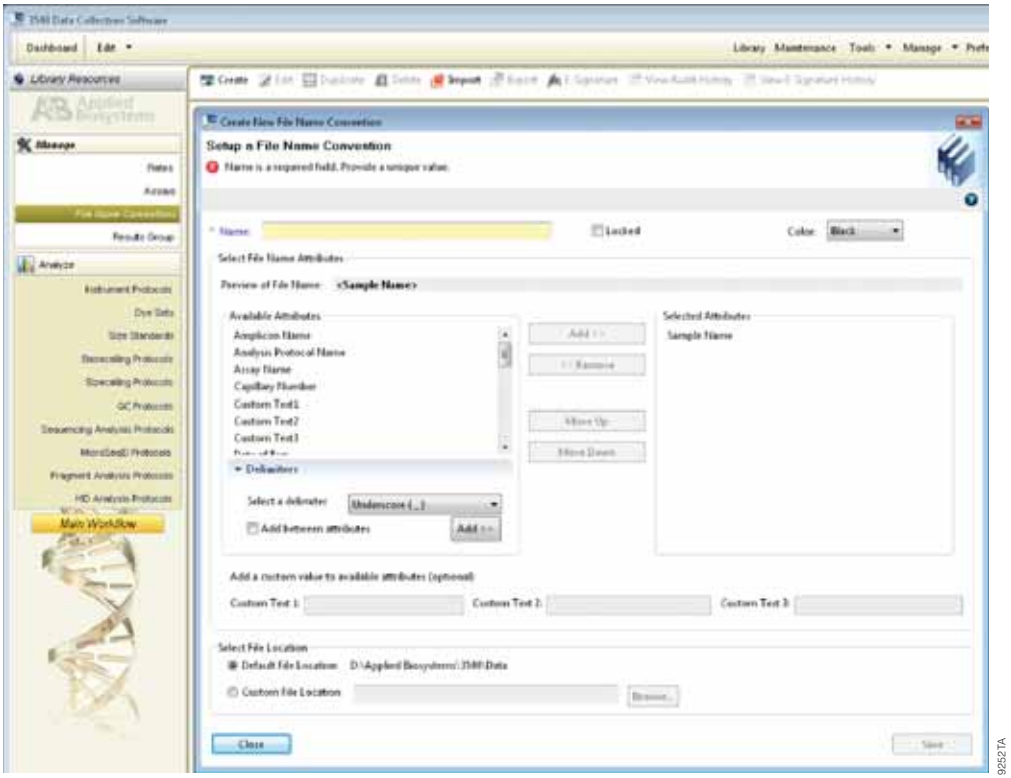

Figure 7. The Create New File Name Convention window.

7. To create a new Results Group (Figure 8), navigate to the Library. Select "Results Group", then select "Create". Alternatively, a previously created Results Groups may be used.

   Select the Results Group Attributes according to laboratory practices, and save with a descriptive name.

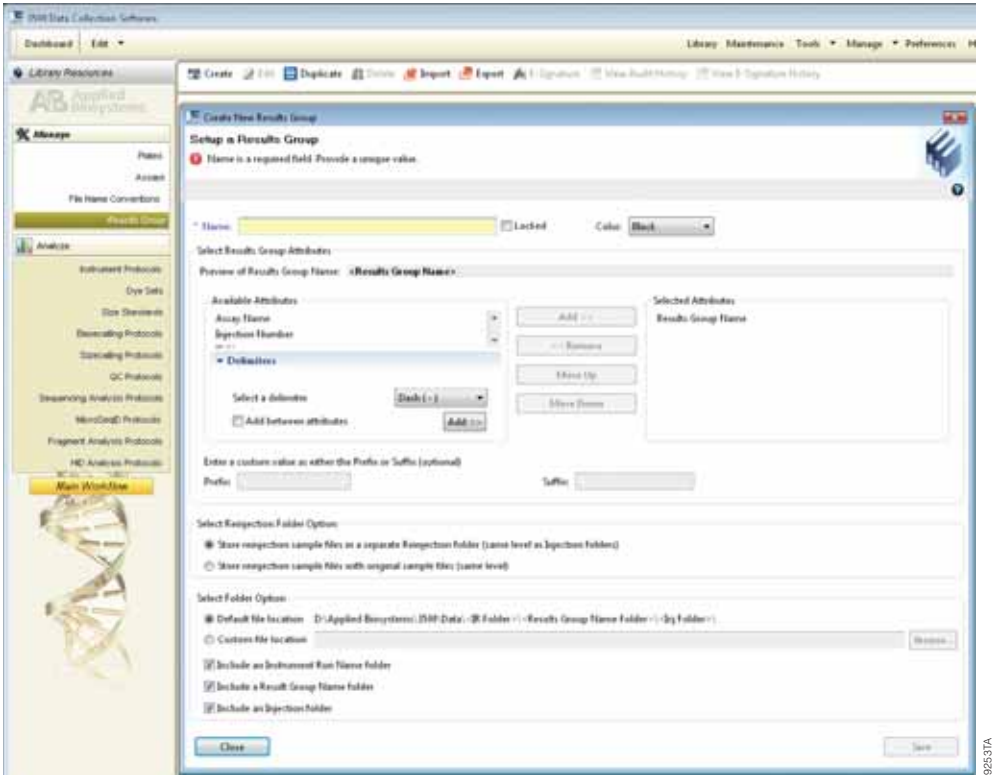

Figure 8. The Create New Results Group window.

8. To create a New Plate, navigate to the Library, and from the Manage menu, select "Plates", then "Create".

9. Assign a descriptive plate name. Select the plate type "HID" from the drop-down menu (Figure 9).

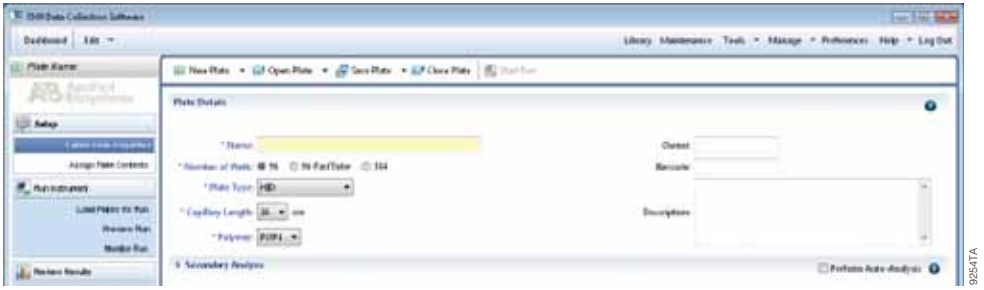

Figure 9. Defining plate properties.

10. Select "Assign Plate Contents" (Figure 10).

11. Assign sample names to wells.

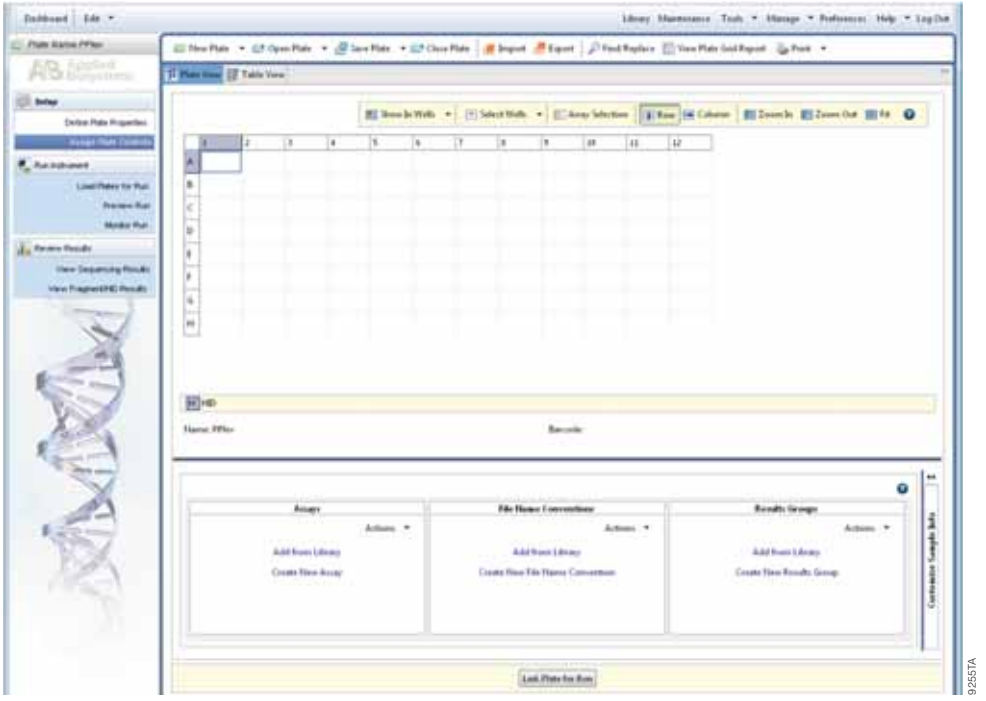

Figure 10. Assigning plate contents.

12. In the lower left portion of the screen, under "Assays", use the Add from Library option to select the Assay created in Step 5 or one previously created. Click on the Add to Plate button, and close the window.

13. Under "File Name Convention", use the Add from Library option to select the File Name Convention created in Step 6 or one previously created. Click on the Add to Plate button, and close the window.

14. Under "Results Groups", use the Add from Library option to select the Results Group created in Step 7 or one previously created. Click on the Add to Plate button, and close the window.

15. Highlight the sample wells, then select the boxes in the Assays, File Name Conventions and Results Groups that pertain to those samples.

16. Select "Link Plate for Run".

17. The Load Plate window will appear. Select "Yes".

18. In the Run Information window (Figure 11), assign a Run Name. Select "Start Run" (not shown).

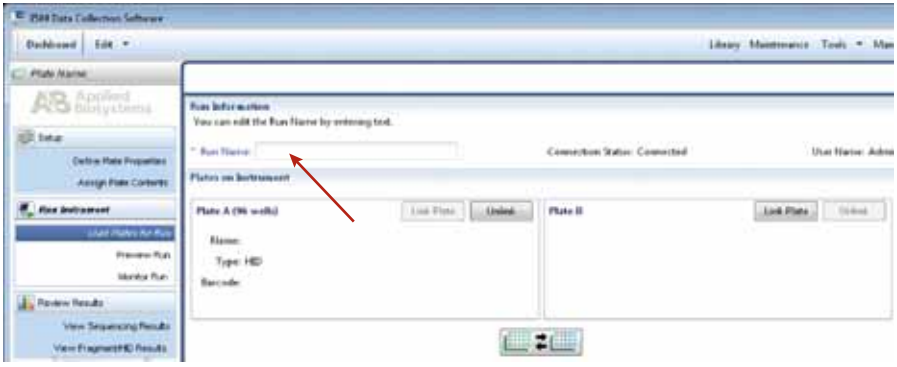

Figure 11. Assigning a run name.

5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, or the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3100 or 3130 capillary array, 36cm
- performance optimized polymer 4 (POP-4® polymer) for the 3100 or 3130
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate (or equivalent) and septa
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, or the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software Version 3.0 (continued)

The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Sample Preparation

1. Thaw the CC5 Internal Lane Standard 500.

   Note: Centrifuge tube briefly to bring contents to the bottom, then vortex for 15 seconds before each use. Do not centrifuge after vortexing, as this may cause the size standard to be concentrated at the bottom of the tube.

2. Prepare a loading cocktail by combining and mixing CC5 Internal Lane Standard 500 and Hi-Di™ formamide as follows:

   [(1.0µl CC5 ILS 500) × (# samples)] + [(10.0µl Hi-Di™ formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of the size standard peaks. Use a volume of CC5 ILS 500 that results in peak heights that are all consistently above the peak amplitude threshold of the orange dye channel determined as part of your internal validation. Keep the volume of formamide at 10.0µl, and adjust the volume added to the wells in Step 4 accordingly.

3. Vortex for 10–15 seconds to mix.

4. Pipet 11µl of formamide/internal lane standard mix into each well.

5. Add 1µl of amplified sample (or 1µl of PowerPlex® ESX 17 Allelic Ladder Mix). Cover wells with appropriate septa.

   Notes:

   1. Instrument detection limits vary; therefore, injection time, injection voltage or the amount of product mixed with loading cocktail may need to be adjusted. Use the Module Manager in the data collection software to modify the injection time or voltage in the run module (see Instrument Preparation below). If the injection time or voltage is reduced, a decreased peak amplitude threshold for the orange channel may be required for proper sizing.
   2. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your internal validation.

6. Centrifuge plate briefly to remove air bubbles from the wells.
7. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

Instrument Preparation

Refer to the instrument user's manual for instructions on cleaning, installing the capillary array, performing a spatial calibration and adding polymer.

Analyze samples as described in the user's manual for the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, and the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0, with the following exceptions.

1. In the Module Manager, select "New". Select "Regular" in the Type drop-down list, and select "HIDFragmentAnalysis36_POP4" in the Template drop-down list. Confirm that the injection time is 5 seconds, the injection voltage is 3kV and the run time is 1,500 seconds. Give a descriptive name to your run module, and select "OK".

   Note: Instrument sensitivities can vary. The injection time and voltage may be adjusted in the Module Manager. A suggested range for the injection time is 3–22 seconds and for the injection voltage is 1–3kV.

2. In the Protocol Manager, select "New". Type a name for your protocol. Select "Regular" in the Type drop-down list, and select the run module you created in the previous step in the Run Module drop-down list. Lastly, select "G5" in the dye-set drop-down list. Select "OK".

3. In the Plate Manager, create a new plate record as described in the instrument user's manual. In the dialog box that appears, select "GeneMapper—Generic" in the Application drop-down list, and select the appropriate plate type (96-well). Add entries in the owner and operator windows, and select "OK".

   Note: If autoanalysis of sample data is desired, refer to the instrument user's manual for instructions.

4. In the GeneMapper plate record, enter sample names in the appropriate cells. Scroll to the right. In the Results Group 1 column, select the desired results group. In the Instrument Protocol 1 column, select the protocol you created in Step 2. Be sure this information is present for each row that contains a sample name. Select "OK".

   Note: To create a new results group, select "New" in the drop-down menu in the Results Group column. Select the General tab, and enter a name. Select the Analysis tab, and select "GeneMapper—Generic" in the Analysis type drop-down list.

5. Place samples in the instrument, and close the instrument doors.

5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, or the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software Version 3.0 (continued)

6. In the spectral viewer, select dye set G5, and confirm that the active dye set is the file generated for the PowerPlex® 5-dye chemistry.

   It is critical to select the correct G5 spectral for the PowerPlex® 5-dye chemistry.

   If the PowerPlex® 5-dye chemistry is not the active dye set, locate the PowerPlex® 5-dye spectral in the List of Calibrations for Dye Set G5, and select "Set".

7. In the run scheduler, locate the plate record that you just created in Steps 3 and 4, and click once on the name to highlight it.

8. Once the plate record is highlighted, click the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples.

9. When the plate record is linked to the plate, the plate graphic will change from yellow to green, and the green Run Instrument arrow becomes enabled.

10. Click on the green Run Instrument arrow on the toolbar to start the sample run.

11. Monitor electrophoresis by observing the run, view, array or capillaries viewer window in the data collection software. Each injection will take approximately 40 minutes.

5.C. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- 310 capillaries, 47cm × 50µm
- performance optimized polymer 4 (POP-4® polymer)
- 10X genetic analyzer buffer with EDTA
- sample tubes and septa
- aerosol-resistant pipette tips
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Sample Preparation

1. Thaw the CC5 Internal Lane Standard 500.

   Note: Centrifuge tube briefly to bring contents to the bottom, then vortex for 15 seconds before each use. Do not centrifuge after vortexing, as this may cause the size standard to be concentrated at the bottom of the tube.

2. Prepare a loading cocktail by combining the CC5 Internal Lane Standard 500 and Hi-Di™ formamide as follows:

   [(1.0µl CC5 ILS 500) × (# samples)] + [(24.0µl Hi-Di™ formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of the size standard peaks. Use a volume of CC5 ILS 500 that results in peak heights that are all consistently above the peak amplitude threshold of the orange dye channel determined as part of your internal validation. If peak heights are too high, we recommend altering the loading cocktail to contain 0.5µl of CC5 ILS 500 and 24.5µl of Hi-Di™ formamide.

3. Vortex for 10–15 seconds to mix.

4. Combine 25.0µl of prepared loading cocktail and 1.0µl of amplified sample (or 1µl of PowerPlex® ESX 17 Allelic Ladder Mix).

   Notes:

   1. Instrument detection limits vary; therefore, injection time, injection voltage or the amount of product mixed with loading cocktail may need to be increased or decreased. Use the Module Manager in the data collection software to modify the injection time or voltage in the run module (see Instrument Preparation below). If the injection time or voltage is reduced, a decreased peak amplitude threshold for the orange channel may be required for proper sizing.
   2. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your internal validation.

5. Centrifuge tubes briefly to remove air bubbles from the wells.

6. Denature samples and ladder by heating at 95°C for 3 minutes, and immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading.

7. Place the tubes in the appropriate autosampler tray.

8. Place the autosampler tray in the instrument, and close the instrument doors.

5.C. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer (continued)

Instrument Preparation

Refer to the instrument user's manual for instructions on cleaning the pump block, installing the capillary, calibrating the autosampler and adding polymer to the syringe.

1. Open the ABI PRISM® 310 Data Collection Software.
2. Prepare a GeneScan® sample sheet as described in the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Enter the appropriate sample information in the Sample Info column.

   For rows containing PowerPlex® ESX 17 Allelic Ladder Mix, insert the word "ladder" in the Sample Info column for the fluorescein, JOE, TMR-ET, CXR-ET and CC5 dyes when using the GeneScan® and Genotyper® software. This information must be entered to successfully analyze your data using the PowerTyper™ ESX 17 Macro.
3. Create a new GeneScan® injection list. Select the appropriate sample sheet by using the drop-down menu.
4. Select the "GS STR POP4 (1ml) G5" Module using the drop-down menu. Change the injection time to 3 seconds and the run time to 28 minutes. Keep the settings for the remaining parameters as shown below:

Inj. Secs:	3
Inj. kV:	15.0
Run kV:	15.0
Run °C:	60
Run Time:	28

(!) You may need to optimize the injection time for individual instruments. Injection times of 2–5 seconds are suggested for samples that contain 0.5ng of template DNA.

Note: Migration of fragments may vary slightly over the course of a long ABI PRISM® 310 Genetic Analyzer run. This may be due to changes in temperature or changes in the column. When analyzing many samples, injections of allelic ladder at different times throughout the run can aid in accurately genotyping samples.

5. Select the appropriate matrix file.
6. To analyze data automatically, select the auto analyze checkbox and the appropriate analysis parameters and size standard. Refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual* for specific information about these options.
7. After loading the sample tray and closing the doors, select "Run" to start the capillary electrophoresis system.
8. Monitor electrophoresis by observing the raw data and status windows. Each sample will take approximately 35 minutes for syringe pumping, sample injection and sample electrophoresis.

6. Data Analysis

6.A. Importing PowerPlex® ESX Panels, Bins and Stutter Text Files into GeneMapper® *ID*-X Software, Version 1.2

To facilitate analysis of data generated with the PowerPlex® ESX 17 System, we have created panels and bins text files to allow automatic assignment of genotypes using GeneMapper® *ID*-X software. We recommend that users receive training from Applied Biosystems on the GeneMapper® *ID*-X software to familiarize themselves with proper operation of the software.

Note: The panels, bins and stutter text files mentioned here are compatible with earlier versions of the GeneMapper® *ID*-X software.

Getting Started

1. To obtain the proper panels, bins and stutter text files for the PowerPlex® ESX 17 System go to: **www.promega.com/resources/tools/genemapper-id-software-panels-and-bin-sets/**
2. Select the PowerPlex® System that you are using, and select "GeneMapper ID-X". Enter your contact information, and select "Submit".
3. Save the PowerPlex_ESX_Panels_IDX_vX.x.txt, PowerPlex_ESX_Bins_IDX_vX.x.txt and PowerPlex_ESX_Stutter_IDX_vX.x.txt files, where "X.x" refers to the most recent version of the panels, bins and stutter text files, to a known location on your computer.

Importing Panels, Bins and Stutter Text Files

1. Open the GeneMapper® *ID*-X software.
2. Select "Tools", then "Panel Manager".
3. Highlight the Panel Manager icon in the upper left navigation pane.
4. Select "File", then "Import Panels".
5. Navigate to the panels file downloaded in the Getting Started Section. Select the file, then "Import".
6. In the navigation pane, highlight the PowerPlex ESX panels folder that you just imported in Step 5.
7. Select "File", then "Import Bin Set".
8. Navigate to the bins text file downloaded in the Getting Started Section. Select the file, then "Import".
9. In the navigation pane, highlight the PowerPlex ESX panels folder that you just imported in Step 5.
10. Select "File", then "Import Marker Stutter". A warning box will appear asking if you want to overwrite current values. Select "Yes".

6.A. Importing PowerPlex® ESX Panels, Bins and Stutter Text Files into GeneMapper® *ID*-X Software, Version 1.2 (continued)

11. Navigate to the stutter file downloaded in the Getting Started Section. Select the file, then "Import".
12. At the bottom of the Panel Manager window, select "OK". This will save the panels, bins and stutter text files and close the window.

6.B. Importing the CC5 ILS 500 Size Standard into GeneMapper® *ID*-X Software, Version 1.2

There are two options when creating a size standard. Use this protocol or the alternative protocol in Section 6.C.

The CC5_ILS_500_IDX.xml file is available for download at: **www.promega.com/resources/tools/genemapper-id-software-panels-and-bin-sets/**

Save the CC5_ILS_500_IDX.xml file to a known location on your computer.

1. Select "Tools", then "GeneMapper ID-X Manager".
2. Select the Size Standard tab.
3. Select "Import".
4. Navigate to the location of the CC5_ILS_500_IDX.xml file on your computer.
5. Highlight the file, then select "Import".
6. Select "Done" to save changes and close the GeneMapper ID-X Manager.

6.C. Creating a Size Standard with GeneMapper® *ID*-X Software, Version 1.2

1. Select "Tools", then "GeneMapper ID-X Manager".
2. Select the Size Standard tab.
3. Select "New".
4. In the Size Standard Editor window (Figure 12), select "GeneMapper ID-X Security Group" as the Security Group. This allows access for all users of the software. Other security groups may be used.
5. Enter a detailed name, such as "CC5_ILS_500_IDX".
6. Choose "Orange" for the Size Standard Dye.
7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Section 9.C, Figure 25.
8. Select "OK".

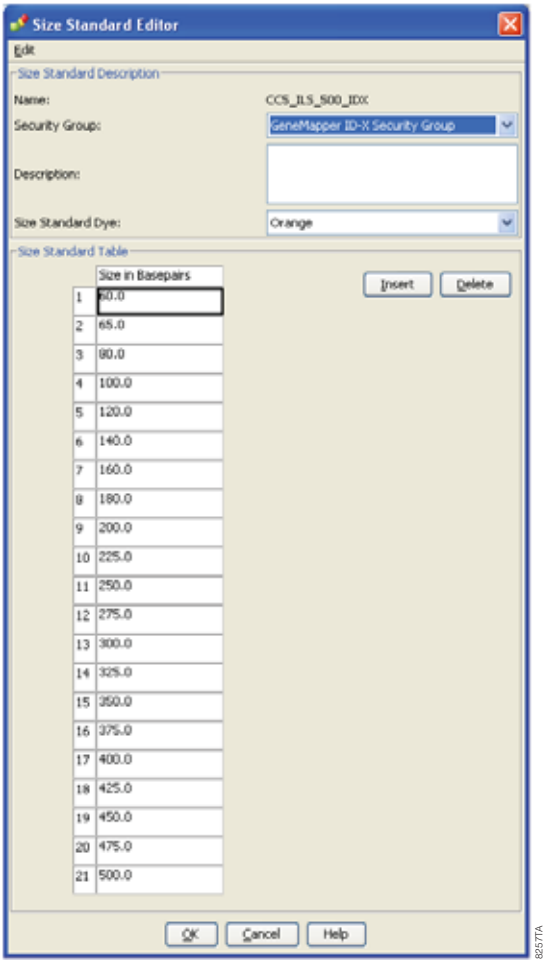

Figure 12. The GeneMapper® *ID*-X Size Standard Editor.

6.D. Creating a Casework Analysis Method with GeneMapper® *ID*-X Software, Version 1.2

These instructions are intended as a guide to start analyzing data in GeneMapper® *ID*-X software. They are not intended as a comprehensive guide for using GeneMapper® *ID*-X software. We recommend that users contact Applied Biosystems for training on the software.

1. Select "Tools", then "GeneMapper ID-X Manager".
2. Select the Analysis Methods tab.
3. Select "New", and a new analysis method dialog box will open.
4. In the Analysis Method Editor window, select "GeneMapper ID-X Security Group" as the Security Group. This allows access for all users of the software. Other security groups may be used.

6.D. Creating a Casework Analysis Method with GeneMapper® *ID*-X Software, Version 1.2 (continued)

5. Enter a descriptive name for the analysis method, such as "PowerPlexESX 17".
6. Select the Allele tab (Figure 13).
7. Select the bins text file that was imported in Section 6.A.
8. Ensure that the "Use marker-specific stutter ratio and distance if available" box is checked.
9. We recommend the values shown in Figure 13 for proper filtering of stutter peaks when using the PowerPlex® ESX 17 System. You may need to optimize these settings. In-house validation should be performed.

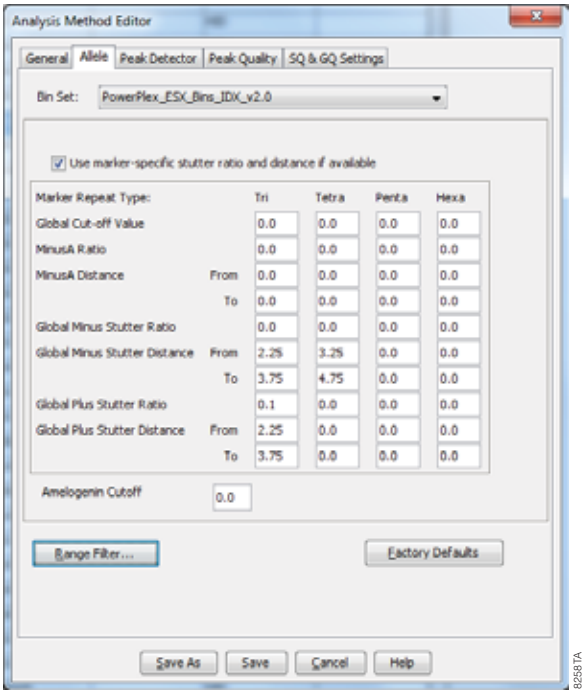

Figure 13. The GeneMapper® *ID*-X Allele tab.

10. Select the Peak Detector tab. Figure 14 shows an example of settings used at Promega. You may need to optimize these settings. In-house validation should be performed.

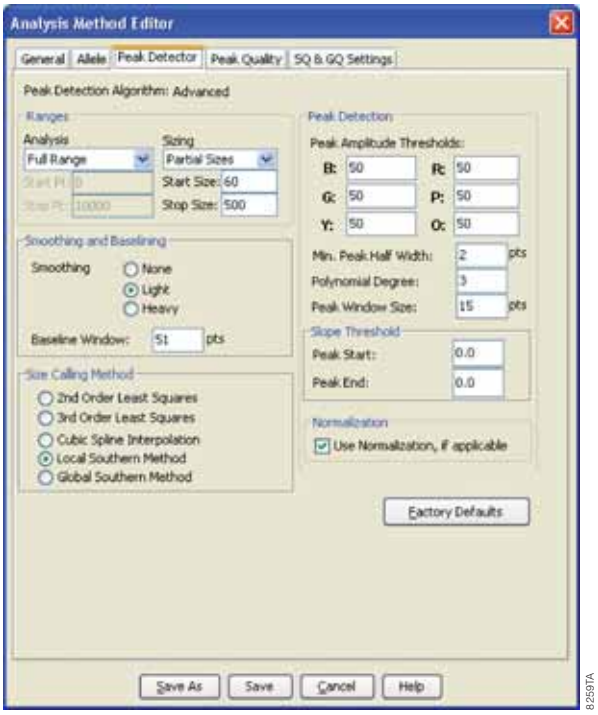

Figure 14. The GeneMapper® *ID*-X Peak Detector tab.

Notes:

1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.
2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Values for peak amplitude thresholds are usually 50–150RFU for data generated on the ABI PRISM® 310, 3100 and 3100-*Avan*t Genetic Analyzers and Applied Biosystems® 3130 and 3130*xl* Genetic Analyzers. For the Applied Biosystems® 3500 and 3500xL Genetic Analyzers, Life Technologies suggests an analysis threshold of 175RFU when using the default injection conditions. However, individual laboratories should determine their peak amplitude thresholds from internal validation studies.
3. Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes. Be sure that all CC5 ILS 500 peaks are consistently above the peak amplitude threshold for the orange dye channel determined as part of your internal validation.
4. The normalization box can be checked regardless of whether normalization was or was not applied during data collection.

6.D. Creating a Casework Analysis Method with GeneMapper® *ID-X* Software, Version 1.2 (continued)

11. Select the Peak Quality tab. You may change the settings for peak quality.

    Note: For Steps 11 and 12, see the GeneMapper® *ID*-X user's manual for more information.

12. Select the SQ & GQ Settings tab. You may change these settings.

13. Select "Save" to save the new analysis method.

14. Select "Done" to exit the GeneMapper ID-X Manager.

Processing Data for Casework Samples

1. Select "File", then "New Project".

2. Select "Edit", then "Add Samples to Project".

3. Browse to the location of the run files. Highlight the desired files, then select "Add to list" followed by "Add".

4. In the Sample Type column, use the drop-down menu to select "Allelic Ladder", "Sample", "Positive Control" or "Negative Control" as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as "Allelic Ladder" in the Sample Type column for proper genotyping.

5. In the Analysis Method column, select the analysis method created previously in this section.

6. In the Panel column, select the panels text file that was imported in Section 6.A.

7. In the Size Standard column, select the size standard that was imported in Section 6.B or created in Section 6.C.

8. If analyzing data from an ABI PRISM® 310 Genetic Analyzer, ensure that the appropriate matrix file is selected in the Matrix column.

9. Select "Analyze" (green arrow button) to start data analysis.

   Note: By default, the software displays the Analysis Requirement Summary, Allelic Ladder Analysis Summary and Analysis Summary windows after quality review by the software. Ensure that all requirements are met as each window appears. If you do not have the Analysis Requirement Summary window activated, you may need to do additional manual troubleshooting.

10. If all analysis requirements are met, the Save Project window will open (Figure 15).

Figure 15. The Save Project window.

11. Enter the project name.
12. Choose the applicable security group from the drop-down menu, then select "OK".

When the analysis is finished, the Analysis Summary screen will appear. We recommend that you review any yellow or red marker header bars in the plots view and handle them according to laboratory standard operating procedures. Navigate to the Genotype tab or Samples tab. To assist the review of any low-quality samples, use the default Data Interpretation plot settings and review the contents in the Quality Value Details table.

The values displayed in the Analysis Method Peak Quality and SQ & GQ Settings tabs are defaults and will affect the quality values displayed in the plot settings. We recommend that you modify the values in these tabs to fit your laboratory's data analysis protocols.

6.E. Creating a Databasing or Paternity Analysis Method with GeneMapper® *ID*-X Software, Version 1.2

These instructions are intended as a guide to start analyzing data in GeneMapper® *ID*-X software. They are not intended as a comprehensive guide for using the GeneMapper® *ID*-X software. We recommend that users contact Applied Biosystems for training on the software.

1. Select "Tools", then "GeneMapper ID-X Manager".
2. Select the Analysis Methods tab.
3. Select "New", and a new analysis method dialog box will open.
4. In the Analysis Method Editor window, select "GeneMapper ID-X Security Group" as the Security Group. This allows access for all users of the software. Other security groups may be used.
5. Enter a descriptive name for the analysis method, such as "PowerPlexESX 17 20% Filter".
6. Select the Allele tab (Figure 16).

7. Select the bins text file that was imported in Section 6.A.

8. We recommend the values shown in Figure 16 for proper filtering of stutter peaks when using the PowerPlex® ESX 17 System. You may need to optimize these settings. In-house validation should be performed.

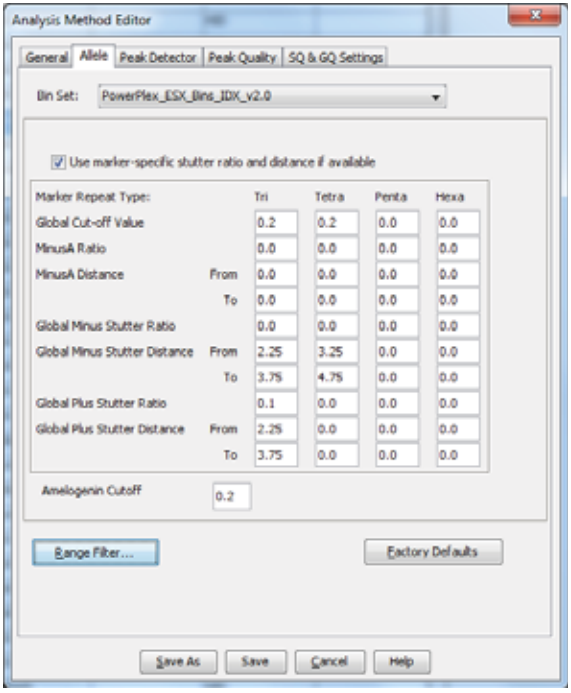

Figure 16. The GeneMapper® *ID*-X Allele tab.

9. Select the Peak Detector tab. Figure 14 shows an example of settings used at Promega. You may need to optimize these settings. In-house validation should be performed.

   Notes:

   1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.
   2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Values for peak amplitude thresholds are usually 50–150RFU on the ABI PRISM® 310, 3100 and 3100-*Avan*t Genetic Analyzers and Applied Biosystems® 3130 and 3130*xl* Genetic Analyzers. For the Applied Biosystems® 3500 and 3500xL Genetic Analyzers, Life Technologies suggests an analysis

threshold of 175RFU when using the default injection conditions. However, individual laboratories should determine their peak amplitude thresholds from internal validation studies.

3. Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes. Be sure that all CC5 ILS 500 peaks are consistently above the peak amplitude threshold for the orange dye channel determined as part of your internal validation.
4. The normalization box can be checked regardless of whether normalization was or was not applied during data collection.

10. Select the Peak Quality tab. You may change the settings for peak quality.

    Note: For Steps 10 and 11, see the GeneMapper® *ID*-X user's manual for more information.

11. Select the SQ & GQ Settings tab. You may change these settings.
12. Select "Save" to save the new analysis method.
13. Select "Done" to exit the GeneMapper ID-X Manager.

Processing Data for Databasing or Paternity Samples

1. Select "File", then "New Project".
2. Select "Edit", then "Add Samples to Project".
3. Browse to the location of run files. Highlight desired files, then select "Add to list" followed by "Add".
4. In the Sample Type column, use the drop-down menu to select "Allelic Ladder", "Sample", "Positive Control" or "Negative Control" as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as "Allelic Ladder" in the Sample Type column for proper genotyping.

   In the Analysis Method column, select the analysis method created previously in this section.
5. In the Panel column, select the panels file that was imported in Section 6.A.
6. In the Size Standard column, select the size standard that was imported in Section 6.B or created in Section 6.C.
7. If analyzing data from an ABI PRISM® 310 Genetic Analyzer, ensure that the appropriate matrix file is selected in the Matrix column.
8. Select "Analyze" (green arrow button) to start data analysis.

   Note: By default, the software displays the Analysis Requirement Summary, Allelic Ladder Analysis Summary and Analysis Summary windows after quality review by the software. Ensure that all requirements are met as each window appears. If you do not have the Analysis Requirement Summary window activated, you may need to do additional manual troubleshooting.

6.E. Creating a Databasing or Paternity Analysis Method with GeneMapper® *ID*-X Software, Version 1.2 (continued)

9. If all analysis requirements are met, the Save Project window will open (Figure 15).
10. Enter the project name.
11. Choose the applicable security group from the drop-down menu, then select "OK".

When the analysis is finished, the Analysis Summary screen will appear. We recommend that you review any yellow or red marker header bars in the plots view and handle them according to laboratory standard operating procedures. Navigate to the Genotype tab or Samples tab. To assist the review of any low-quality samples, use the default Data Interpretation plot settings and review the contents in the Quality Value Details table.

The values displayed in the Analysis Method Peak Quality and SQ & GQ Settings tabs are defaults and will affect the quality values displayed in the plot settings. We recommend that you modify the values in these tabs to fit your laboratory's data analysis protocols.

6.F. Importing PowerPlex® ESX Panels and Bins Text Files into GeneMapper® *ID* Software, Version 3.2

To facilitate analysis of data generated with the PowerPlex® ESX 17 System, we have created panels and bins text files to allow automatic assignment of genotypes using GeneMapper® *ID* software, version 3.2. We recommend that users of GeneMapper® *ID* software, version 3.2, complete the *Applied Biosystems GeneMapper®* ID *Software Human Identification Analysis Tutorial* to familiarize themselves with proper operation of the software. For GeneMapper® *ID* software, version 3.1, users we recommend upgrading to version 3.2.

For analysis using GeneMapper® *ID* software, version 3.2, you will need the proper panels and bins text files: PowerPlex_ESX_Panels_vX.x.txt and PowerPlex_ESX_Bins_vX.x.txt files, where "X.x" refers to the most recent version of the panels and bins text files.

Getting Started

1. To obtain the panels and bins text files for the PowerPlex® ESX 17 System go to: **www.promega.com/resources/tools/genemapper-id-softwarepanels-and-bin-sets/**
2. Select the PowerPlex® System that you are using, and select "GeneMapper ID". Enter your contact information, and select "Submit".
3. Save the PowerPlex_ESX_Panels_vX.x.txt and PowerPlex_ESX_Bins_vX.x.txt files to a known location on your computer.

Importing Panels and Bins Text Files

These instructions loosely follow the Applied Biosystems GeneMapper® *ID* software tutorial, pages 1–4.

1. Open the GeneMapper® *ID* software, version 3.2.
2. Select "Tools", then "Panel Manager".
3. Highlight the Panel Manager icon in the upper left navigation pane.
4. Select "File", then "Import Panels".
5. Navigate to the panels text file downloaded in the Getting Started section above. Select the file, then "Import".
6. In the navigation pane, highlight the PowerPlex ESX panels folder that you just imported in Step 5.
7. Select "File", then "Import Bin Set".
8. Navigate to the bins text file downloaded in the Getting Started section above. Select the file, then "Import".
9. At the bottom of the Panel Manager window, select "OK". The Panel Manager window will close automatically.

6.G. Importing the CC5 ILS 500 Size Standard into GeneMapper® *ID* Software, Version 3.2

There are two options when creating a size standard. Use this protocol or the alternative protocol in Section 6.H.

The CC5_ILS_500.xml file is available for download at:
www.promega.com/resources/tools/genemapper-id-software-panels-and-bin-sets/

Save the CC5_ILS_500.xml file to a known location on your computer.

1. Select "Tools", then "GeneMapper Manager".
2. Select the Size Standard tab.
3. Select "Import".
4. Browse to the location of the CC5_ILS_500.xml file.
5. Highlight the file, then select "Import".
6. Select "Done" to save changes and exit the GeneMapper Manager.

6.H. Creating a Size Standard with GeneMapper® *ID* Software, Version 3.2

1. Select "Tools", then "GeneMapper Manager".
2. Select the Size Standard tab.
3. Select "New".
4. Select "Basic or Advanced" (Figure 17). The type of analysis method selected must match the type of analysis method created earlier. Select "OK".

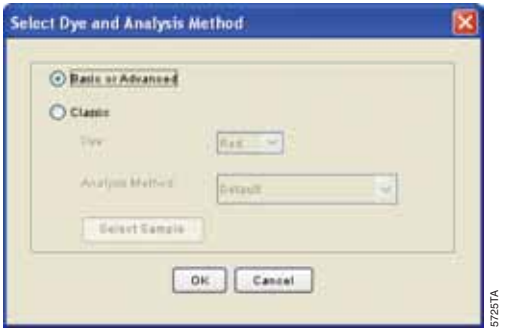

Figure 17. The Select Dye and Analysis Method window.

5. Enter a detailed name, such as "CC5 ILS 60 to 500", in the Size Standard Editor (Figure 18).

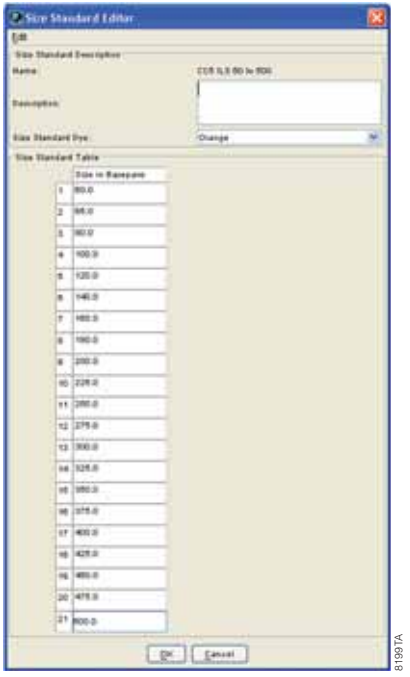

Figure 18. The Size Standard Editor.

6. Choose "Orange" for the Size Standard Dye.

7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Section 9.C, Figure 25.

8. Select "OK".

6.I. Creating a Casework Analysis Method with GeneMapper® *ID* Software, Version 3.2

These instructions loosely follow the Applied Biosystems GeneMapper® *ID* software tutorial, pages 5–11.

1. Select "Tools", then "GeneMapper Manager".

2. Select the Analysis Methods tab.

3. Select "New", and a new analysis method dialog box will open.

4. Select "HID", and select "OK".

   Note: If you do not see the HID option, you do not have the GeneMapper® *ID* software. Contact Applied Biosystems.

5. Enter a descriptive name for the analysis method, such as "PowerPlexESX 17".

6. Select the Allele tab (Figure 19).

7. Select the bins text file that was imported in Section 6.F.

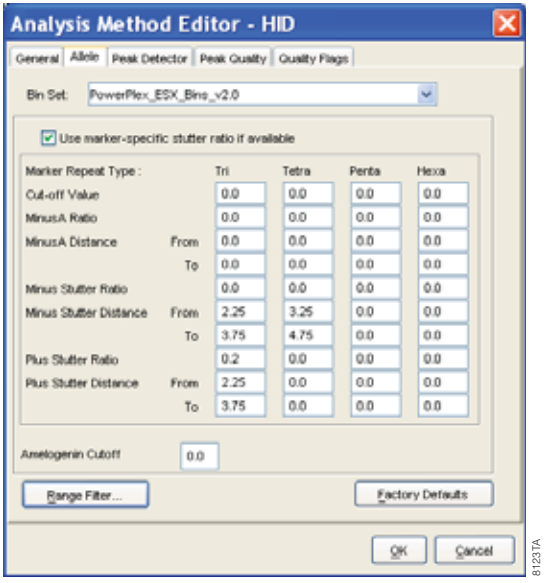

Figure 19. The GeneMapper® *ID* Allele tab.

6.I. Creating a Casework Analysis Method with GeneMapper® *ID* Software, Version 3.2 (continued)

8. Ensure that the "Use marker-specific stutter ratio if available" box is checked.

9. Enter the values shown in Figure 19 for proper filtering of stutter peaks when using the PowerPlex® ESX 17 System. For an explanation of the proper usage and effects of these settings, refer to the Applied Biosystems user bulletin titled "*Installation Procedures and New Features for GeneMapper* ID *Software 3.2*".

   Note: Some of these settings have been optimized and are different from the recommended settings in the user bulletin.

10. Select the Peak Detector tab. We recommend the settings shown in Figure 20.

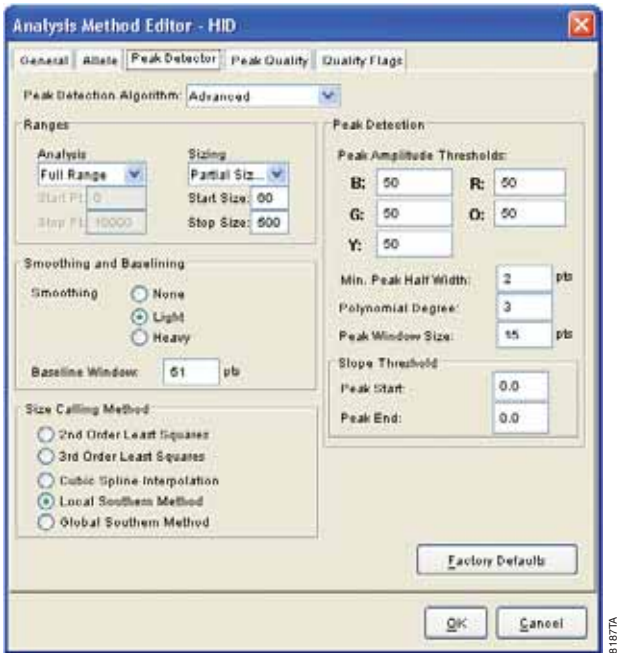

Figure 20. The GeneMapper® *ID* Peak Detector tab.

Notes:

1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.

2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Values for peak amplitude thresholds are usually 50–150RFU and should be determined by individual laboratories.

3. Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes. Be sure that all CC5 ILS 500 peaks are consistently above the peak amplitude threshold for the orange dye channel determined as part of your internal validation.

11. Select the Peak Quality tab. You may change the settings for peak quality.

    Note: For Steps 11 and 12, see the GeneMapper® *ID* user's manual for more information.

12. Select the Quality Flags tab. You may change these settings.

13. Select "OK" to save your settings.

Processing Data for Casework Samples

1. Select "File", then "New Project".

2. Select "Edit", then "Add Samples to Project".

3. Browse to the location of the run files. Highlight desired files, then select "Add to list" followed by "Add".

4. In the Sample Type column, use the drop-down menu to select "Ladder", "Sample", "Positive Control" or "Negative Control" as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as "Ladder" in the Sample Type column for proper genotyping.

5. In the Analysis Method column, select the analysis method created previously in this section.

6. In the Panel column, select the panels file that was imported in Section 6.F.

7. In the Size Standard column, select the size standard that was imported in Section 6.G or created in Section 6.H.

8. If analyzing data from an ABI PRISM® 310 Genetic Analyzer, ensure that the appropriate matrix file is selected in the Matrix column.

9. Select "Analyze" (green arrow button) to start data analysis.

6.J. Creating a Databasing or Paternity Analysis Method with GeneMapper® *ID* Software, Version 3.2

1. Select "Tools", then "GeneMapper Manager".
2. Select the Analysis Methods tab.
3. Select "New", and a new analysis method dialog box will open.
4. Select "HID", and select "OK".

   Note: If you do not see the HID option, you do not have the GeneMapper® *ID* software. Contact Applied Biosystems.
5. Enter a descriptive name for the analysis method, such as "PowerPlexESX 17_20%filter".
6. Select the Allele tab (Figure 21).
7. Select the bins text file that was imported in Section 6.F.
8. Ensure that the "Use marker-specific stutter ratio if available" box is checked.
9. Enter the values shown in Figure 21 for proper filtering of peaks when using the PowerPlex® ESX 17 System. For an explanation of the proper usage and effect of these settings, refer to the Applied Biosystems user bulletin titled "*Installation Procedures and New Features for GeneMapper* ID *Software* 3.2".

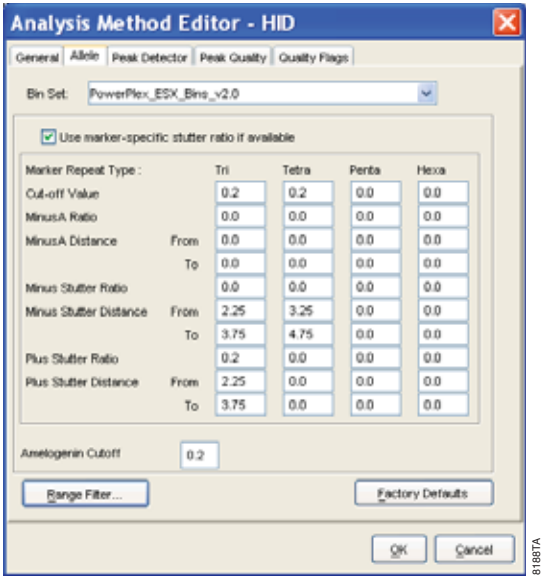

Figure 21. The GeneMapper® *ID* Allele tab with settings for using a 20% peak filter.

10. Select the Peak Detector tab. We recommend the settings shown in Figure 20.

    Notes:

    1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.
    2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Values for peak amplitude thresholds are usually 50–150RFU. Individual laboratories should determine their peak amplitude thresholds from internal validation studies.
    3. Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes. Be sure that all CC5 ILS 500 peaks are consistently above the peak amplitude threshold for the orange dye channel determined as part of your internal validation.

11. Select the Peak Quality tab. You may change the settings for peak quality.

    Note: For Steps 11 and 12, see the GeneMapper® *ID* user's manual for more information.

12. Select the Quality Flags tab. You may change these settings.

13. Select "OK" to save your settings.

Processing Data for Databasing or Paternity Samples

1. Select "File", then "New Project".
2. Select "Edit", then "Add Samples to Project".
3. Browse to the location of the run files. Highlight desired files, then select "Add to list" followed by "Add".
4. In the Sample Type column, use the drop-down menu to select "Ladder", "Sample", "Positive Control" or "Negative Control" as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as "Ladder" in the Sample Type column for proper genotyping.
5. In the Analysis Method column, select the analysis method created previously in this section.
6. In the Panel column, select the panels text file that was imported in Section 6.F.
7. In the Size Standard column, select the size standard that was imported in Section 6.G or created in Section 6.H.
8. If analyzing data from an ABI PRISM® 310 Genetic Analyzer, ensure that the appropriate matrix file is selected in the Matrix column.
9. Select "Analyze" (green arrow button) to start the data analysis.

6.K. Sample Analysis Using the GeneScan® Software and Windows® Operating Systems

1. Analyze data using the GeneScan® software.
2. Review the raw data for one or more sample runs. Highlight the sample file name, then in the Sample menu, select "raw data". Move the cursor so that the crosshair is on the baseline to the right of the large primer peak (before the first internal lane standard peak [orange]). Use the X-value number shown at the bottom left of the window for the start position in the analysis parameters.
3. The recommended analysis parameters are shown in Figure 22.

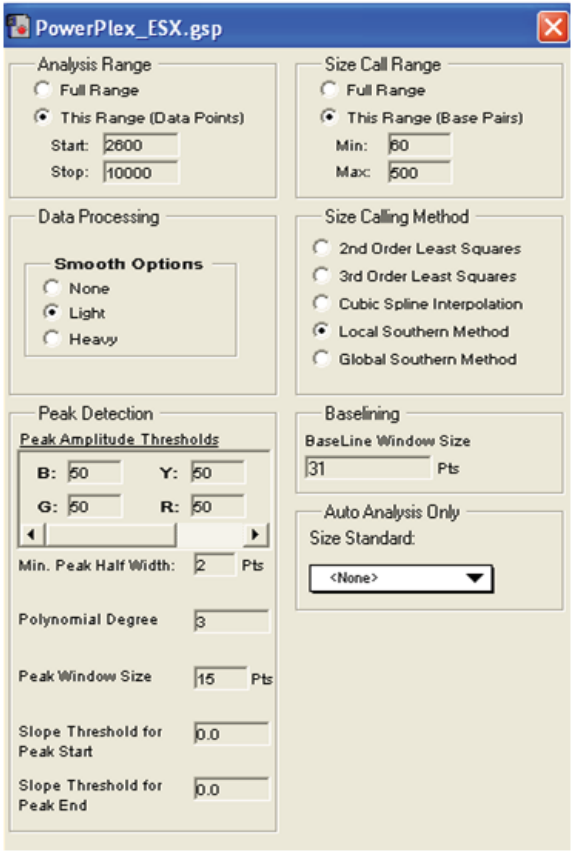

Figure 22. The Analysis Parameters window. The start point of the analysis range, which will vary, is defined in Section 6.K, Step 2.

Notes:

1. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Values for peak amplitude thresholds are usually 50–150RFU and should be determined by individual laboratories.
2. Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes. Be sure that all CC5 ILS 500 peaks are consistently above the peak amplitude threshold for the orange dye channel determined as part of your internal validation.

4. The analysis parameters can be saved in the Params folder; in most installations this is located at:
   C:\AppliedBio\Shared\Analysis\Sizecaller\Params
5. Apply the stored analysis parameters file to the samples.
6. Assign a new size standard. Select a sample file, and highlight the arrow next to size standard. Select "define new". Assign the size standard peaks of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases. Store the size standard in the Size Standards folder at: C:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards
7. Apply the size standard file to the samples, then analyze the sample files. See Section 6.L for additional information about the use of the PowerTyper™ ESX 17 Macro and Genotyper® software.

Notes:

1. Peak heights outside the linear range of the instrument may generate artifact peaks due to instrument saturation (i.e., overloading the sample). Bleedthrough (pull-ups) from one color to another may be observed. Saturated signal also may appear as two peaks (split peak).
2. If peak heights are not within the linear range of detection of the instrument, the ratio of stutter peaks to real allele peaks increases, and allele designations become difficult to interpret. The balance of peak heights also may appear less uniform.
3. There can be variation between instruments regarding the relative fluorescence levels detected using the same sample. Furthermore, different instruments vary in the relative efficiency of color detection, affecting the dye color-to-dye color balance.

6.L. Sample Analysis Using the Genotyper® Software and PowerTyper™ ESX 17 Macro

To facilitate analysis of data generated with the PowerPlex® ESX 17 System, we have created a file to allow automatic assignment of genotypes using the Genotyper® software. After samples are amplified, detected using the ABI PRISM® 310 or 3100 Genetic Analyzer, and analyzed using the GeneScan® software, sample files can be imported into the Genotyper® program and analyzed using the PowerTyper™ ESX 17 Macro.

The PowerTyper™ ESX Macros are available for download at:
www.promega.com/resources/tools/powertyper-macros/

The PowerTyper™ ESX 17 Macro is used in conjunction with Macintosh® Genotyper® software, version 2.5, and Windows NT® Genotyper® software, version 3.6, or later. The Genotyper® software must be installed on your computer before the PowerTyper™ ESX 17 Macro can be used.

Be certain the Sample Info (Macintosh® computers) or Color Info (Windows NT® operating systems) column for each lane containing allelic ladder mix contains the word "ladder". The macro uses the word "ladder" to identify the sample file(s) containing allelic ladder. Sample info can be added or modified after importing into the PowerTyper™ ESX 17 Macro. Highlight the sample, then select "show dye/lanes window" in the Views menu.

1. Transfer the PowerTyper_ESX_17 Macro file to a designated location on your computer hard drive.
2. Open the Genotyper® software, then the PowerTyper_ESX_17 Macro file. For questions about the Genotyper® software, refer to the *Genotyper® Analysis Software User's Manual*.
3. In the File menu, select "Import", and import the GeneScan® project or sample files to be analyzed. Import the fluorescein, JOE, TMR-ET, CXR-ET and CC5 dyes.

   Note: To select the dye colors to be imported, select "Set Preferences" in the Edit menu.
4. Double-click on the Check ILS macro. The macros are listed at the bottom left corner of the active window. A plots window will be displayed to show the internal lane standard (i.e., CC5 ILS 500) in the CC5 (orange) channel. Scroll down to view and confirm that the internal lane standard fragment sizes are correct. If necessary, re-analyze samples using the GeneScan® software and redefine internal lane standard fragments.

   Notes:

   1. The software uses one ladder sample to determine allele sizes. The macro uses the first ladder sample imported for allele designations.
   2. The Macintosh® version of the Genotyper® software displays the fragment sizes for the internal lane standard. However, it displays the raw data for the electropherogram instead of the analyzed data.

5. For casework, double-click on the POWER macro. The POWER macro identifies alleles in the ladder sample and calculates offsets for all loci. This process may take several minutes. When completed, a plots window will open to display the allelic ladders (i.e., D18S51, D21S11, TH01, D3S1358 and Amelogenin).

   Alternatively, for databasing or paternity, double-click on the POWER 20% Filter macro. This macro has a higher level of filtering than the standard POWER macro to reduce the need for manual editing of peak labels. The POWER 20% Filter should not be used if mixtures may exist.

6. Double-click on the Allelic Ladders macro. A plots window will open to display the fluorescein allelic ladders (i.e., D18S51, D21S11, TH01, D3S1358 and Amelogenin), JOE allelic ladders (i.e., D16S539, D2S1338, D1S1656 and D10S1248), TMR-ET allelic ladders (i.e., FGA, D8S1179, vWA and D22S1045) and CXR-ET allelic ladders (i.e., SE33, D19S433, D12S391 and D2S441). Confirm that the correct allele designations were assigned to the allelic ladders (Figure 24 in Section 6.N and Table 5 in Section 9.A).

   The software uses one ladder sample to determine allele sizes. The macro uses the first ladder sample imported for allele designations. If the POWER macro is run a second time, the software will use the second ladder; if the POWER macro is run a third time, the software will use the third ladder, etc., until all ladders in the project are used. If an allelic ladder fails to be analyzed or if many off-ladder alleles are found in the samples, samples should be re-analyzed using another ladder from the project.

   Note: FGA alleles 50.2 and 48.2 exhibit high stutter. The PowerTyper™ ESX 17 Macro will label these stutter peaks as alleles 49.2 and 47.2.

7. Double-click on the Display Blue Data macro to display fluorescein data for all sample injections. Scroll down to observe and edit as needed.

8. Double-click on the Display Green Data macro to display JOE data for all sample injections. Scroll down to observe and edit as needed.

9. Double-click on the Display Yellow Data macro to display TMR-ET data for all sample injections. Scroll down to observe and edit as needed.

10. Double-click on the Display Red Data macro to display CXR-ET data for all sample injections. Scroll down to observe and edit as needed.

6.L. Sample Analysis Using the Genotyper® Software and PowerTyper™ ESX 17 Macro (continued

11. Create the appropriate table by selecting the PowerTable, Make Allele Table or Make CODIS Table macro. The three available table formats are shown below. The PowerTable option allows up to four alleles per sample file. Additional information such as low peak signal or high peak signal is also included. The Allele Table and CODIS Table options include only two alleles per locus. If more than two alleles are present at a locus, the smallest alleles identified are included. The Allele Table format displays the categories (loci) in columns, while the CODIS table format displays the categories in rows. These tables can be customized to fit needs. To save data in tables, go to the Table drop-down menu, highlight "Export to File..." and save the file with the desired name and location. The saved file can be viewed and analyzed using Microsoft Excel®.

12. Save the analyzed data. Go to the File menu, and select "Save as".

The PowerTyper™ ESX 17 Macro is a Genotyper® file and can be overwritten if "Save" is used instead of "Save as".

PowerTable Format

Sample Info	Sample Comment	Category	Peak 1	Peak 2	Peak 3	Peak 4	Over-flow	Low Signal	Satura-tion	Edited Label	Edited Row

Allele Table Format

Sample Info	Category Allele 1	Category Allele 2	Category Allele 1	Category Allele 2	Category Allele 1	Category Allele 2	Category Allele 1	Category Allele 2

CODIS Table Format

Sample Info	Category	Peak 1	Peak 2

6.M. Controls

1. Observe the results for the negative control. Using the protocols defined in this manual, the negative control should be devoid of amplification products.

2. Observe the results for the 2800M Control DNA. Compare the 2800M DNA allelic repeat sizes with the locus-specific allelic ladder. The expected 2800M DNA allele designations for each locus are listed in Table 6 (Section 9.A).

6.N. Results

Representative results of the PowerPlex® ESX 17 System are shown in Figure 23. The PowerPlex® ESX 17 Allelic Ladder Mix is shown in Figure 24.

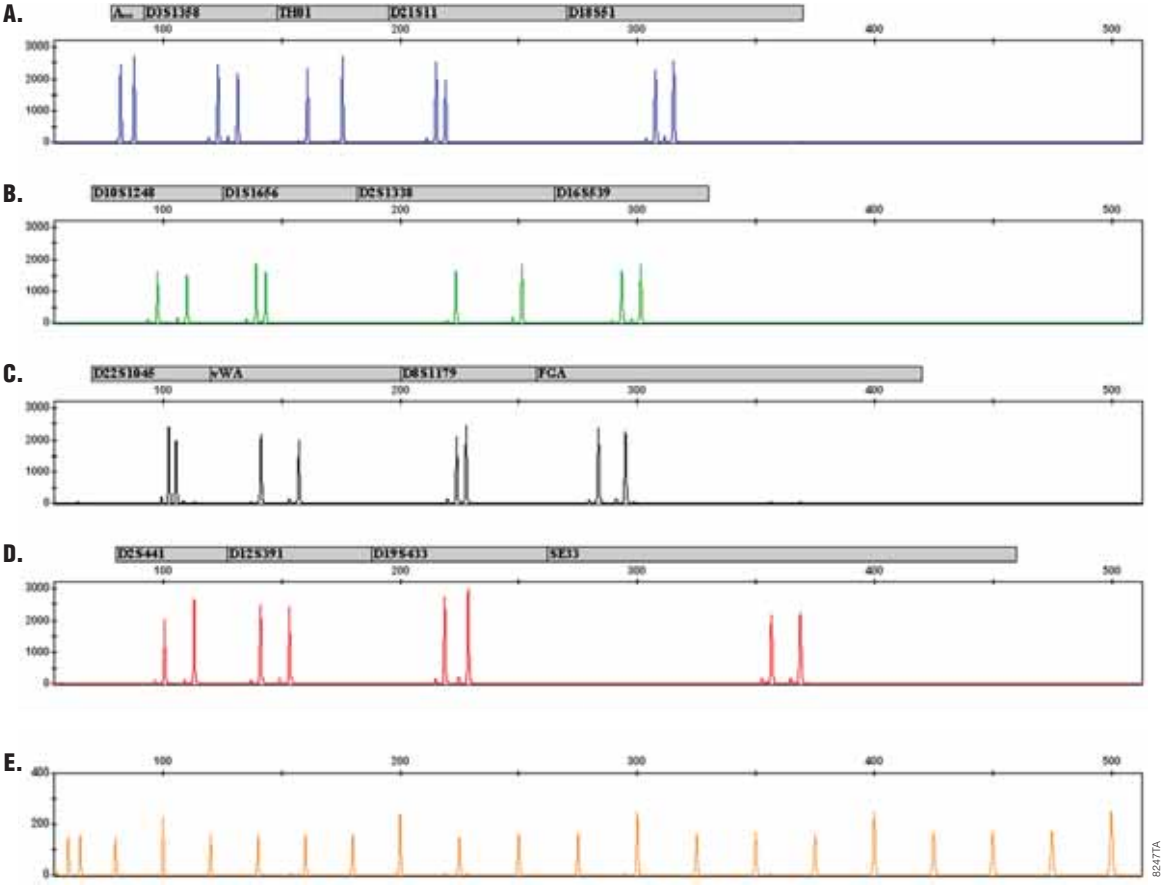

Figure 23. The PowerPlex® ESX 17 System. A single-source template DNA (0.5ng) was amplified using the PowerPlex® ESX 17 System. Amplification products were mixed with CC5 Internal Lane Standard 500 and analyzed with an Applied Biosystems® 3130 Genetic Analyzer using a 3kV, 5-second injection. Results were analyzed using GeneMapper® *ID* software, version 3.2. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: Amelogenin, D3S1358, TH01, D21S11 and D18S51. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: D10S1248, D1S1656, D2S1338 and D16S539. **Panel C.** An electropherogram showing the peaks of the TMR-ET-labeled loci: D22S1045, vWA, D8S1179 and FGA. **Panel D.** An electropherogram showing the peaks of the CXR-ET-labeled loci: D2S441, D12S391, D19S433 and SE33. **Panel E.** An electropherogram showing the 60bp to 500bp fragments of the CC5 Internal Lane Standard 500.

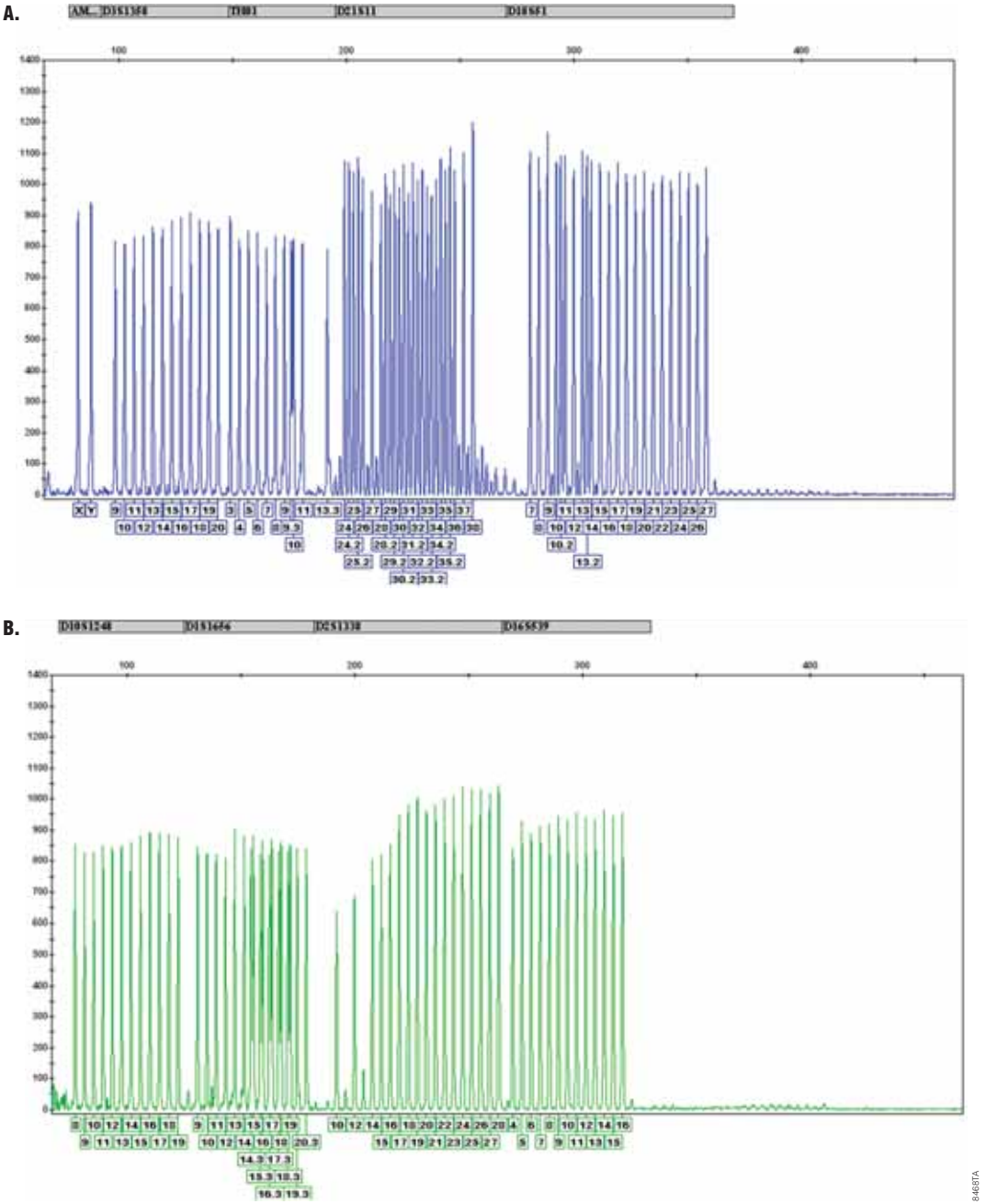

Figure 24. The PowerPlex® ESX 17 Allelic Ladder Mix. The PowerPlex® ESX 17 Allelic Ladder Mix was analyzed with an Applied Biosystems® 3130 Genetic Analyzer using a 3kV, 5-second injection. The sample file was analyzed with the GeneMapper® *ID* software, version 3.2, and PowerPlex® ESX panels and bins text files. **Panel A.** The fluorescein-labeled allelic ladder components and their allele designations. **Panel B.** The JOE-labeled allelic ladder components and their allele designations. **Note:** Panels C and D are shown on the next page.

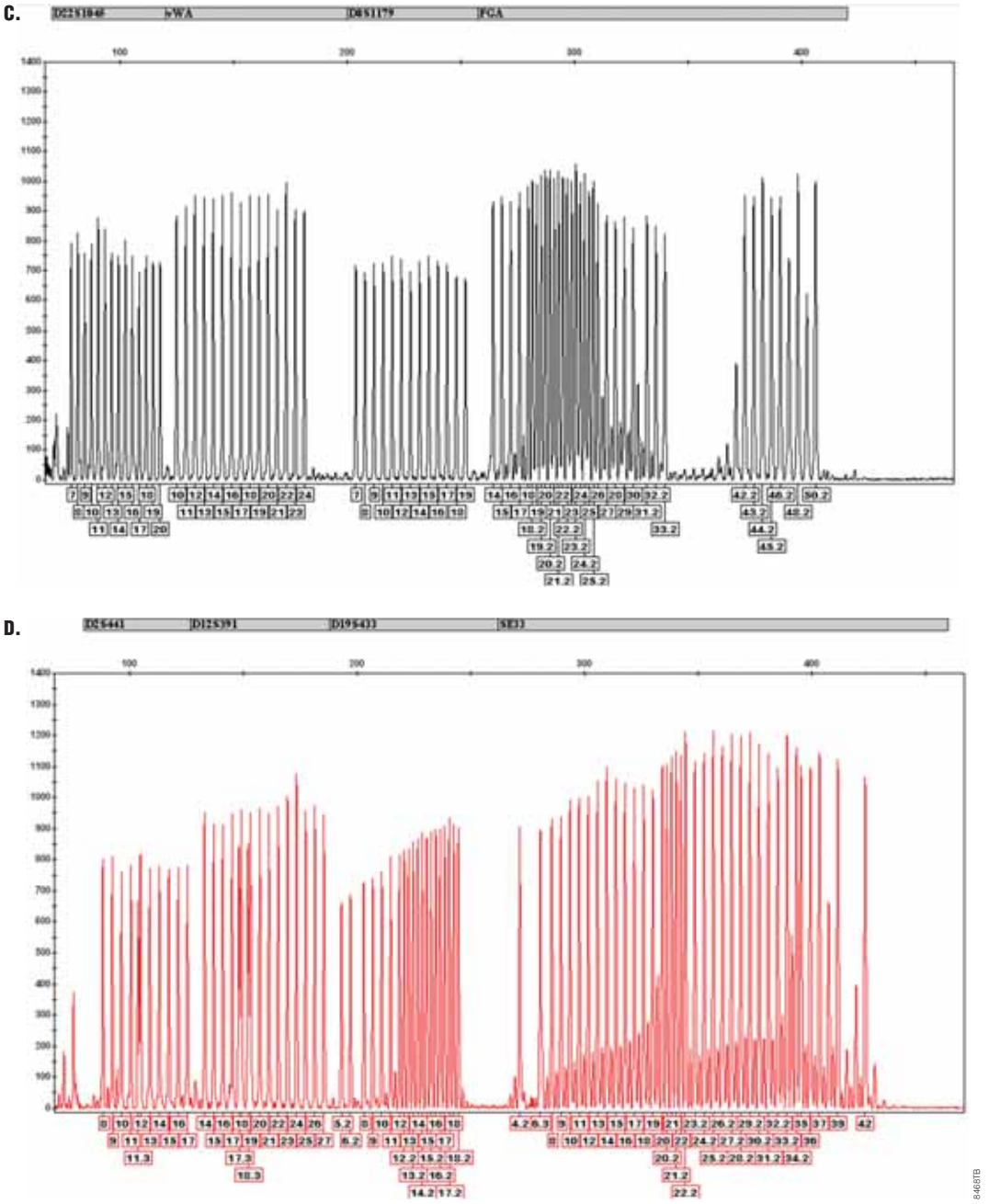

Figure 24. The PowerPlex® ESX 17 Allelic Ladder Mix (continued). The PowerPlex® ESX 17 Allelic Ladder Mix was analyzed with an Applied Biosystems® 3130 Genetic Analyzer using a 3kV, 5-second injection. The sample file was analyzed with the GeneMapper® *ID* software, version 3.2, and PowerPlex® ESX panels and bins text files. **Panel C.** The TMR-ET-labeled allelic ladder components and their allele designations. **Panel D.** The CXR-ET-labeled allelic ladder components and their allele designations. **Note:** Panels A and B are shown on the previous page.

6.N. Results (continued)

Artifacts and Stutter

Stutter products are a common amplification artifact associated with STR analysis (15,16). Stutter products are often observed one repeat unit below the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percent stutter. The pattern and intensity of stutter may differ slightly between primer sets for the same loci. Increased stutter often is associated with D22S1045, as it is a trinucleotide repeat marker.

The highest stutter observed at each locus is used in the PowerPlex® ESX panels text files for locus-specific filtering in the GeneMapper® *ID* software, version 3.2, GeneMapper® *ID-X* software and PowerTyper™ ESX 17 Macro for Genotyper® software.

In addition to stutter peaks, the following low-level artifact peaks may be observed with the PowerPlex® ESX 17 System loci.

Locus	Instrument	Artifact Sizes[1]
D21S11, D2S441, D1S1656 and SE33	ABI PRISM® 310 and Applied Biosystems® 3130 Genetic Analyzers	n–2; n+2[2]
Amelogenin	ABI PRISM® 310 and Applied Biosystems® 3130 Genetic Analyzers	n–1[3]
		65–71 bases Artifact migrates before Amelogenin
	ABI PRISM® 310 Genetic Analyzer only	72–73 bases (male and female samples)[4]
		77–78 bases (male samples only)[4]
	Applied Biosystems® 3130 Genetic Analyzer only	76 bases (male and female samples)[4]
		85 bases (male samples only)[4]
D10S1248	ABI PRISM® 310 and Applied Biosystems® 3130 Genetic Analyzers	60–64 bases Artifact migrates before D10S1248[5]

[1]The artifacts listed here are DNA-dependent.

[2]Two bases below and above the true allele peak, respectively.

[3]The n–1 artifact is more noticeable with high template amounts and allele peak heights.

[4]These variably sized peaks on the ABI PRISM® 310 and Applied Biosystems® 3130 Genetic Analyzers may represent double-stranded DNA derived from the Amelogenin amplicon (double-stranded DNA is known to migrate faster than single-stranded DNA on capillary electrophoresis instruments). This artifact is only seen with high peak heights for the X and Y alleles.

[5]Low-level, DNA-dependent artifact is noticeable only with high template amounts and allele peak heights. The artifact migrates approximately 13–17 bases in front of the smallest allele (allele 8) in D10S1248. This peak may be above or below analysis threshold, depending on the sensitivity of the capillary electrophoresis instrument.

7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **genetic@promega.com**

7.A. Amplification and Fragment Detection

This section provides information about general amplification and detection. For questions about direct amplification, see Sections 7.B and 7.C.

Symptoms	Causes and Comments
Faint or absent allele peaks	Impure template DNA. Because of the small amount of template used, this is rarely a problem. Depending on the DNA extraction procedure used and sample source, inhibitors might be present in the DNA sample.
	Insufficient template. Use the recommended amount of template DNA if available.
	The PowerPlex® ESX 5X Master Mix was not vortexed well before use. Vortex the 5X Master Mix for 15 seconds before dispensing into the PCR amplification mix.
	An air bubble formed at the bottom of the reaction tube. Use a pipette to remove the air bubble, or centrifuge the reactions briefly before thermal cycling.
	High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K^+, Na^+, Mg^{2+} or EDTA from the DNA sample can negatively affect PCR. A change in pH also may affect PCR. Store DNA in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA), TE^{-4} buffer with 20µg/ml glycogen or nuclease-free water.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl. Decreasing the reaction volume may result in suboptimal performance.
	Thermal cycler, plate or tube problems. Review the thermal cycling protocols in Section 4. We have not tested other reaction tubes, plates or thermal cyclers. Calibrate the thermal cycler heating block if necessary.
	Primer concentration was too low. Use the recommended primer concentration. Vortex the PowerPlex® ESX 17 10X Primer Pair Mix for 15 seconds before use.
	Samples were not denatured completely. Heat-denature samples for the recommended time, then cool on crushed ice or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool the samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing.
	Poor capillary electrophoresis injection (CC5 ILS 500 Pro peaks also affected). Re-inject the sample. Check the 310 instrument syringe pump system for leakage.
	Poor capillary electrophoresis injection (CC5 ILS 500 Pro peaks also affected). Check the laser power.
	Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples.

7.A. Amplification and Fragment Detection (continued)

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.
	Artifacts of STR amplification. Amplification of >0.5ng template can result in a higher number of artifact peaks. Use less template DNA. See Section 6.N for additional information on stutter and artifacts.
	Samples were not denatured completely. Heat denature samples for the recommended time, and cool on crushed ice or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool the samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing.
	Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis. Appearance of "shadow" peaks migrating in front of the main peaks, especially if the shadow peaks are separated by the same distant as the main peaks in a heterozygote, can indicate the presence of double-stranded DNA due to incomplete denaturation or post-injection re-annealing.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. Be sure to perform the 45-minute extension step at 60°C after thermal cycling (Section 4).
	CE-related artifacts ("spikes"). Minor voltage changes or urea crystals passing by the laser can cause "spikes" or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject samples to confirm.
	Incorrect G5 spectral was active. Re-run samples, and confirm that the PowerPlex® 5-dye G5 spectral is set for G5. See instructions on instrument preparation in Section 5.
	Pull-up or bleedthrough. Pull-up can occur when peak heights are too high or if a poor or incorrect matrix is applied to the samples. • For the ABI PRISM® 310 Genetic Analyzer, generate a new matrix, and apply it to the samples. For the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers, perform a new spectral calibration, and re-run the samples. • Instrument sensitivities can vary. Optimize the injection conditions. See Section 5.
	CE-related artifacts (contaminants). Contaminants in the water used with the instrument or to dilute the 10X genetic analyzer buffer may generate peaks in the fluorescein and JOE channels Use autoclaved deionized water; change vials and wash buffer reservoir.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	Repeat sample preparation using fresh formamide. Long-term storage of amplified samples in formamide can result in artifacts.
	The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than one week.
	Maintain instrumentation on a daily or weekly basis, as recommended by the manufacturer.
Allelic ladder not running the same as samples	Allelic ladder and primer pair mix were not compatible. Ensure that the allelic ladder is from the same kit as the primer pair mix.
	Poor-quality formamide. Use only Hi-Di™ formamide when analyzing samples.
	Be sure the allelic ladder and samples are from the same instrument run.
	Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature or the CE column over time. Use a different injection of allelic ladder to determine sizes.
	Poor injection of allelic ladder. Include more than one ladder per instrument run.
Peak height imbalance	Excessive amount of DNA. Amplification of >0.5ng of template can result in an imbalance, with smaller loci showing more product than larger loci. Use less template.
	Degraded DNA sample. DNA template was degraded, and larger loci showed diminished yield. Repurify template DNA.
	Insufficient template DNA. Use the recommended amount of template DNA if available. Stochastic effects can occur when amplifying low amounts of template.
	Miscellaneous balance problems. Thaw the 10X Primer Pair Mix and 5X Master Mix completely, and vortex for 15 seconds before use. Do not centrifuge the 10X Primer Pair Mix after mixing. Calibrate thermal cyclers and pipettes routinely.
	PCR amplification mix prepared in Section 4 was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into the reaction tubes or plate.
	Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance.

7.B. Direct Amplification of DNA from Storage Card Punches

The following information is specific to direct amplification. For information about general amplification and detection, see Section 7.A.

Symptoms	Causes and Comments
Faint or absent allele peaks	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl to overcome inhibitors present in FTA® cards and PunchSolution™ Reagent. Decreasing the reaction volume may result in suboptimal performance.
	DNA was not accessible on nonlytic material. Pretreat nonFTA materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins.
	Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.
	Poor sample transfer to storage card or variable sampling from storage card. Take punches from a different portion of the card. Increasing cycle number can improve low peak heights.
	Too much sample in the reaction. Use one or two 1.2mm storage card punches. Follow the manufacturer's recommendations when depositing sample onto the storage card. With storage cards, reducing the reaction volumes below 25µl may result in amplification failure.
	Amplification was inhibited when using more than one storage card punch with blood. Use only one 1.2mm storage card punch with blood.
	Make sure that the PCR amplification mix also contained AmpSolution™ Reagent. Omission of AmpSolution™ Reagent from amplification reactions will result in amplification failure.
	Active PunchSolution™ Reagent carried over into the amplification reaction when using nonFTA card punches. Ensure that the heat block was set at 70°C and samples were incubated for 30 minutes until dry. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent. We have not tested longer incubation times.
	Inactive PunchSolution™ Reagent. Thaw the PunchSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.
Faint or absent peaks for the positive control reaction	If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. • Do not include a blank punch in the positive control reaction. Presence of a blank punch may inhibit amplification of 2800M Control DNA. • Optimize the amount of 2800M Control DNA for your thermal cycling conditions and laboratory preferences.

Symptoms	Causes and Comments
Extra peaks visible in one or or all color channels	Punch was contaminated. Take punches from blank paper between samples.
	Amplification of processed punches with high amounts of DNA can result in artifact peaks due to overamplification, resulting in saturating signal on the CE instrument. We recommend one 1.2mm punch per 25µl reaction. Use of a larger punch size or a smaller reaction volume may result in overamplification and signal saturation. If the signal is saturated, repeat the amplification with a smaller punch, a larger reaction volume or reduced cycle number.
	Amplification of excess template for a given cycle number can result in overloading of the capillary upon electrokinetic injection. The presence of excess DNA in the capillary makes it difficult to maintain the DNA in a denatured single-stranded state. Some single-stranded DNA renatures and becomes double-stranded. Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis and appears as "shadow" peaks migrating in front of the main peaks. If this occurs at a heterozygous locus, it is sometimes possible to see two "shadow" peaks that differ in size from one another by approximately the same distance as the single-stranded alleles.
	Artifacts of STR amplification. Direct amplification of >20ng of template can result in a higher number of artifact peaks. Use the recommended punch size and number of punches. Optimize the cycle number. Do not reduce the reaction volume below 25µl. See Section 6.N for additional information on stutter and artifacts.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. • Be sure to perform a 45-minute extension step at 60°C after thermal cycling (Section 4). • Decrease cycle number. • Increase the final extension time.
Peak height imbalance	Excessive amount of DNA. Amplification of >20ng of template can result in an imbalance with smaller loci showing more product than larger loci. • Use one or two 1.2mm punches from a storage card containing a buccal sample or one 1.2mm punch from a storage card containing whole blood. Follow the manufacturer's recommendations when depositing sample onto the storage card. • Decrease cycle number.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl to overcome inhibitors present in FTA® cards and PunchSolution™ Reagent. Decreasing the reaction volume can result in suboptimal performance.

7.B. Direct Amplification of DNA from Storage Card Punches (continued)

Symptoms	Causes and Comments
Peak height imbalance (continued)	Amplification was inhibited when using more than one storage card punch with blood. Use only one 1.2mm storage card punch with blood.
	Active PunchSolution™ Reagent carried over into the amplification reaction. Larger loci are most susceptible to carryover and will drop out before the smaller loci. • Ensure that the heat block was set at 70°C and samples were incubated for 30 minutes until dry. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent. • Using a smaller amplification reaction volume may compromise performance when using 10µl of PunchSolution™ Reagent. Reducing the PunchSolution™ Reagent volume may improve results for reactions with reduced amplification volumes. Optimization and validation are required.
	Inactive PunchSolution™ Reagent. Thaw the PunchSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze; avoid multiple freeze-thaw cycles, as this may reduce activity.
	Carryover of excess PunchSolution™ Reagent into amplification reaction. We recommend treating one 1.2mm nonFTA card punch with 10µl of PunchSolution™ Reagent, and using one punch per 25µl amplification reaction. Use of a smaller amplification reaction volume may compromise performance if using 10µl of PunchSolution™ Reagent. Reducing the PunchSolution™ Reagent volume may improve results when using a reduced amplification reaction volume. Laboratory optimization and validation is required.
Extreme variability in sample-to-sample peak heights	There can be significant individual-to-individual variability in the deposition of cells onto a punch, resulting in peak height variability between samples. The PunchSolution™ Kit increases the recovery of amplifiable DNA from samples but does not normalize the amount of DNA present.

7.C. Amplification of DNA from Swabs

The following information is specific to amplification of DNA from swabs after pretreatment using the SwabSolution™ Kit. For information about general amplification and detection, see Section 7.A.

Symptoms	Causes and Comments
Faint or absent allele peaks	Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.
	Inactive SwabSolution™ Reagent. Thaw the SwabSolution™ Reagent completely in a 37°C water bath, and mix by gentle inversion. Store the SwabSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.

Symptoms	Causes and Comments
Faint or absent allele peaks (continued)	Active SwabSolution™ Reagent carried over into the amplification reaction. Ensure that the heat block is heating to 70°C (90°C if using a 2.2ml, Square-Well Deep Well Plate) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete reagent inactivation. Do not use an incubator set at 70°C to incubate tubes or plates; heat transfer is inefficient and will result in poor performance. Only use a heat block to maintain efficient heat transfer. We have tested 60-minute incubation times and observed no difference in performance compared to a 30-minute incubation.
	Make sure that the PCR amplification mix also contained AmpSolution™ Reagent. Omission of AmpSolution™ Reagent from amplification reactions will result in amplification failure.
Faint or absent peaks for the positive control reaction	If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. Due to the reduced cycle numbers used with swab extracts, it is necessary to increase the mass of 2800M Control DNA to obtain a profile. We recommend 5ng of 2800M Control DNA per 25µl amplification reaction. This mass of DNA should be reduced if the cycle number is increased and decreased if the cycle number is increased. Increase or decrease by twofold the mass of 2800M Control DNA for every one-cycle decrease or increase, respectively.
Extra peaks visible in one or all color channels	Swab extract was contaminated. Assemble a reaction containing the swab extract prepared from a blank swab, or assemble a reaction where the SwabSolution™ Reagent is processed and incubated as a blank without a swab.
	Artifacts of STR amplification. Amplification of swab extracts with high DNA concentrations can result in artifact peaks due to overamplification, resulting in saturated signal on the CE instrument. We recommend 2µl of swab extract per 25µl reaction. Using more than 2µl in a 25µl reaction or using 2µl with a smaller reaction volume may result in overamplification and signal saturation. If signal is saturated, repeat the amplification with less swab extract or a reduced cycle number.
	Amplification of excess template for a given cycle number resulted in overloading of the capillary upon electrokinetic injection. In addition to signal saturation, excess DNA in the capillary is difficult to maintain in a denatured single-stranded state. Some single-stranded DNA renatures and becomes double-stranded. Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis and appears as “shadow” peaks migrating in front of the main peaks. If this occurs at a heterozygous locus it is possible to observe the presence of two “shadow” peaks that differ in size by approximately the same distance as the single-stranded alleles.

7.C. Amplification of DNA from Swabs (continued)

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. • Be sure to perform the 20-minute extension step at 60°C after thermal cycling (Section 4). • Decrease cycle number. • Increase the final extension time.
Peak height imbalance	Excess DNA in the amplification reaction can result in locus-to-locus imbalance within a dye channel such that the peak heights at the smaller loci are greater than those at the larger loci (ski-slope effect). Use less swab extract, or reduce the cycle number.
	Active SwabSolution™ Reagent carried over into the amplification reaction. Larger loci are most susceptible to reagent carryover and will drop out before the smaller loci. Ensure that the heat block is heating to 70°C (90°C if using 2.2ml, Square-Well Deep Well Plates) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete reagent inactivation. Do not use an incubator set at 70°C to incubate tubes or plates; heat transfer is inefficient and will result in poor performance. Only use a heat block to maintain efficient heat transfer.
	Inactive SwabSolution™ Reagent. Thaw the SwabSolution™ Reagent completely in a 37°C water bath, and mix by gentle inversion. Store the SwabSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not re-freeze, as this may reduce activity.
	DNA was not accessible on nonlytic material. Pretreat swabs with SwabSolution™ Reagent to ensure that DNA is liberated from cellular proteins.
Extreme variability in sample-to-sample peak heights	There can be significant individual-to-individual variability in cell deposition onto buccal swabs. This will appear as variability in peak heights between swab extracts. The extraction process maximizes recovery of amplifiable DNA from buccal swabs but does not normalize the amount of DNA present. If variability is extreme, quantitate the DNA using a fluorescence-based double-stranded DNA quantitation method or qPCR-based quantitation method. The quantitation values can be used to normalize input template amounts to minimize variation in signal intensity.
	DNA was not accessible on nonlytic material. Pretreat swabs with SwabSolution™ Reagent to ensure that DNA is liberated from cellular proteins.

Symptoms	Causes and Comments
Stutter peaks not filtered	Stutter file was not imported into the Panel Manager when the panels and bin text files were imported.
	Be sure that the "Use marker-specific stutter ratio and distance if available" box is checked.
	Stutter distance was not defined in the analysis method Allele tab.
Samples in the project not analyzed	Analysis Requirement Summary window was not active, and there was an analysis requirement that was not met. Turn on Analysis Requirement Summary in the Options menu, and correct the necessary analysis requirements to continue analysis.
Edits in label edit viewer cannot be viewed	To view edits made to a project, the project must first be saved. Close the plot view window, go back to the main GeneMapper® *ID*-X page and save the project. Display the plot window again, then view the label edit table.
Marker header bar for some loci are gray	When an edit is made to a locus, the quality flags and marker header bar automatically change to gray. To change the GQ and marker header bar for a locus to green, override the GQ in the plot window.
Alleles not called	To analyze samples with GeneMapper® *ID*-X software, at least one allelic ladder must be defined.
	An insufficient number of CC5 ILS 500 fragments was defined. Be sure to define at least two CC5 ILS 500 fragments smaller than the smallest sample peak or allelic ladder peak and at least two CC5 ILS 500 fragments larger than the largest sample peak or allelic ladder peak. In this instance, the allelic ladder would have failed the allelic ladder quality check.
	Run was too short, and larger peaks in ILS were not captured. Not all CC5 ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
	The GeneMapper® *ID*-X software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze, as described in Section 6.D or 6.E.
	Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.
	The allelic ladder was not identified as an allelic ladder in the Sample Type column.
	The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.

7.D. GeneMapper® *ID*-X Software (continued)

Symptoms	Causes and Comments
Off-ladder alleles (continued)	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
	Incorrect polymer used. Use of a polymer other than POP-4® polymer may change migration of the fragments. Alleles may migrate outside of the panel range established using POP-4® polymer.
Size standard not called correctly	Starting data point was incorrect for the partial range chosen in Section 6.E. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.
	Extra peaks in size standard. Open the Size Match Editor. Highlight the extra peak, select "Edit" and select "delete size label". Select "auto adjust sizes".
	Run was too short, and larger peaks in ILS were not captured. Not all CC5 ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
Peaks in size standard missing	If peaks are below threshold, decrease the peak amplitude threshold in the analysis method for the orange channel to include peaks or increase the volume of CC5 ILS 500 used in Section 5.
	If peaks are low-quality, redefine the size standard for the sample to skip these peaks.
Significantly raised baseline	• Poor spectral calibration for the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers. Perform a new spectral calibration, and re-run the samples. • Poor matrix for the ABI PRISM® 310 Genetic Analyzer. Re-run and optimize the matrix. Make sure that the matrix applied was generated on the same instrument.
	Incorrect G5 spectral was active. Re-run samples, and confirm that the PowerPlex® 5-dye G5 spectral is set for G5. See instructions for instrument preparation in Section 5.

7.E. GeneMapper® *ID* Software

Symptoms	Causes and Comments
Alleles not called	To analyze samples with GeneMapper® *ID* software, the analysis parameters and size standard must both have "Basic or Advanced" as the analysis type. If they are different, an error is obtained.
	To analyze samples with GeneMapper® *ID* software, at least one allelic ladder must be defined.
	An insufficient number of CC5 ILS 500 fragments was defined. Be sure to define at least two CC5 ILS 500 fragments smaller than the smallest sample peak or allelic ladder peak and at least two CC5 ILS 500 fragments larger than the largest sample peak or allelic ladder peak.

Symptoms	Causes and Comments
Alleles not called (continued)	Run was too short, and larger peaks in ILS were not captured. Not all CC5 ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples using an allelic ladder from the same run.
	The GeneMapper® *ID* software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze as described in Section 6.I or 6.J.
	Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.
	The allelic ladder was not identified as an allelic ladder in the Sample Type column.
	The wrong analysis type was chosen for the analysis method. Be sure to use the HID analysis type.
	The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
	Incorrect polymer used. Use of a polymer other than POP-4® polymer may change migration of the fragments. Alleles may migrate outside of the panel range established using POP-4® polymer.
Size standard not called correctly	Starting data point was incorrect for the partial range chosen in Section 6.K. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.
	Extra peaks in advanced mode size standard. Open the Size Match Editor. Highlight the extra peak, select "Edit" and select "delete size label". Select "auto adjust sizes".
	Run was too short, and larger peaks in ILS were not captured. Not all CC5 ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
Peaks in size standard missing	If peaks were below threshold, decrease the peak amplitude threshold in the analysis method for the orange channel to include peaks or increase the volume of CC5 ILS used in Section 5.
	If peaks were low-quality, redefine the size standard for the sample to skip these peaks.

7.E. GeneMapper® *ID* Software (continued)

Symptoms	Causes and Comments
Error message: "Either panel, size standard, or analysis method is invalid"	The size standard and analysis method were not in the same mode ("Classic" vs. "Basic or Advanced"). Be sure both files are set to the same mode, either Classic or Basic or Advanced mode.
No alleles called, but no error message appears	Panels text file was not selected for sample. In the Panel column, select the appropriate panels text file for the STR system that was used.
	No size standard was selected. In the Size Standard column, be sure to select the appropriate size standard.
	Size standard was not correctly defined, or size peaks were missing. Redefine size standard to include only peaks present in your sample. Terminating analysis early or using short run times will cause larger ladder peaks to be missing. This will cause your sizing quality to be flagged as "red", and no allele sizes will be called.
Error message: "Both the Bin Set used in the Analysis Method and the Panel must belong to the same Chemistry Kit"	The bins text file assigned to the analysis method was deleted. In the GeneMapper Manager, select the Analysis Methods tab, and open the analysis method of interest. Select the Allele tab, and select an appropriate bins text file.
	The wrong bins text file was chosen in the analysis method Allele tab. Be sure to choose the appropriate bins text file, as shown in Figure 19.
Red bar appears during analysis of samples, and the following error message appears when data are displayed: "Some selected sample(s) do not contain analysis data. Those sample(s) will not be shown".	If none of the samples had matrices applied when run on the ABI PRISM® 310 Genetic Analyzer, no data will be displayed. Apply a matrix file during analysis in the GeneMapper® *ID* software and re-analyze.
Error message after attempting to import panels and bins text files: "Unable to save panel data: java.SQLEException: ORA-00001: unique constraint (IFA.CKP_NNN) violated".	There was a conflict between different sets of panels and bins text files. Check to be sure that the bins are installed properly. If not, delete all panels and bins text files, and re-import files in a different order.
Allelic ladder peaks labeled off-ladder	GeneMapper® *ID* software was not used, or microsatellite analysis settings were used instead of HID analysis settings. GeneMapper® software does not use the same algorithms as GeneMapper® *ID* software and cannot correct for sizing differences using the allelic ladder. Promega recommends using GeneMapper® *ID* software to analyze PowerPlex® reactions. If using GeneMapper® *ID* software, version 3.2, be sure that the analysis method selected is an HID method. This can be verified by opening the analysis method using the GeneMapper Manager, then selecting the General tab. The analysis type cannot be changed. If the method is not HID, it should be deleted and a new analysis method created.

7.F. PowerTyper™ ESX 17 Macro

Symptoms	Causes and Comments
File does not open on your computer	Genotyper® software was not installed. Be certain that the Genotyper® software, version 2.5 (Macintosh®) or version 3.6 or higher (Windows NT®), is installed.
	Incorrect version of Genotyper® software. The PowerTyper™ ESX 17 Macro will not work with Genotyper® software versions prior to version 2.5 (Macintosh®) or 3.6 (Windows NT®).
Error message: "Could not complete the "Run Macro" command because no dye/lanes are selected"	Allelic ladder sample files were not identified. Be certain the Sample Info or Color Info column for each lane containing PowerPlex® ESX 17 Allelic Ladder Mix contains the word "ladder". The macro uses the word "ladder" to identify sample files containing allelic ladder.
	All five dye colors were not imported. For Genotyper® software, versions 2.5 and 3.6 or higher, set preferences (in the Edit menu) to import fluorescein, JOE, TMR-ET, CXR-ET and CC5 data.
Error message: "Could not complete the "Run Macro" command because the labeled peak could not be found"	Peak heights for one or more alleles in the allelic ladder sample file were below 150RFU. The allelic ladder categories are defined as having a minimum peak height of 150RFU. If peak heights of ladder alleles are below 150RFU, the software will not be able to locate the allele peak. Re-run the allelic ladder using more sample or longer injection time to assure peak heights above 150RFU.
	CE spikes in the allelic ladder sample were identified as alleles by the macro. Use a different injection of allelic ladder.
	Allelic ladder data were not compatible with the PowerTyper™ file used. Confirm that the PowerTyper™ Macro file matches the allelic ladder being used.
	The base-pair size of alleles in the allelic ladder are outside of the defined category range. Be sure internal lane standard fragments are correctly sized. Redefine internal lane standard fragments, and re-analyze the sample using GeneScan® software. Compare the size of the smallest allele in the allelic ladder with the base-pair size and range listed in the categories for the same alleles. If necessary, increase the category start range (in the category window), and save the macro under a new name.
	Allelic ladder peaks were too high, causing stutter peaks to be called as allele peaks. Use a shorter injection time, decrease the amount of allelic ladder used or re-analyze the allelic ladder sample using increased peak amplitude thresholds in the GeneScan® analysis parameters.
	Allelic ladder data were not compatible with the PowerTyper_ESX_17 Macro file used. Confirm that the PowerTyper_ESX_17 Macro file matches the allelic ladder being used.
The plots window or allele table does not display all data	The macros were not run in the proper order. Use the POWER or POWER 20% Filter macro option.
	All five dye colors were not imported. For Genotyper® software, versions 2.5 and 3.6 or higher, set preferences (in the Edit menu) to import fluorescein, JOE, TMR-ET, CXR-ET and CC5 data.

7.F. PowerTyper™ ESX 17 Macro (continued)

Symptoms	Causes and Comments
The Check ILS macro displays an empty plot window	All five dye colors were not imported. For Genotyper® software, versions 2.5 and 3.6 or higher, set preferences (in the Edit menu) to import fluorescein, JOE, TMR-ET, CXR-ET and CC5 data.
Off-ladder peaks	Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature or the CE column over time. Use a different injection of allelic ladder to determine sizes in the PowerTyper™ ESX 17 Macro. Do not use the first injection on a new column for the allelic ladder sample.
	The base-pair size of alleles was incorrect because incorrect fragment sizes were assigned to the internal lane standard. Confirm that internal lane standard fragment sizes are assigned correctly. Re-analyze the sample using GeneScan® software, and redefine the internal lane standard fragments.

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9. Appendix

9.A. Advantages of Using the Loci in the PowerPlex® ESX 17 System

The loci included in the PowerPlex® ESX 17 System (Tables 4 and 5) were selected because they meet the recommendations of the European Network of Forensic Science Institutes (ENFSI). The PowerPlex® ESX 17 System amplifies all ENFSI core loci plus SE33 in a single reaction. Table 6 lists the PowerPlex® ESX 17 System alleles revealed in commonly available standard DNA templates.

Terminal nucleotide addition (17,18) occurs when *Taq* DNA polymerase adds a nucleotide, generally adenine, to the 3´ ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact band one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. We have modified primer sequences and added a final extension step of 60°C for 45 minutes (19) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

Table 4. The PowerPlex® ESX 17 System Locus-Specific Information.

STR Locus	Label	Chromosomal Location[1]	Repeat Sequence[2] 5´fi 3´
D18S51	Fluorescein	18q21.33 (59.1Mb)	AGAA (20)
D21S11	Fluorescein	21q21.1 (19.476Mb)	TCTA Complex (20)
TH01	Fluorescein	11p15.5 (2.149Mb)	AATG (20)
D3S1358	Fluorescein	3p21.31 (45.557Mb)	TCTA Complex
Amelogenin[3]	Fluorescein	Xp22.1–22.3 and Y	NA
D16S539	JOE	16q24.1 (84.944Mb)	GATA
D2S1338	JOE	2q35 (218.705Mb)	TGCC/TTCC
D1S1656	JOE	1q42 (228.972Mb)	TAGA Complex
D10S1248	JOE	10q26.3 (130.567Mb)	GGAA
FGA	TMR-ET	4q28 (155.866Mb)	TTTC Complex (20)
D8S1179	TMR-ET	8q24.13 (125.976Mb)	TCTA Complex (20)
vWA	TMR-ET	12p13.31 (5.963Mb)	TCTA Complex (20)
D22S1045	TMR-ET	22q12.3 (35.779Mb)	ATT
SE33	CXR-ET	6q14 (89.043Mb)	AAAG Complex
D19S433	CXR-ET	19q12 (35.109Mb)	AAGG Complex
D12S391	CXR-ET	12p12 (12.341Mb)	AGAT/AGAC Complex
D2S441	CXR-ET	2p14 (68.214Mb)	TCTA

[1]Information about chromosomal location of these loci can be found in references 21 and 22 and at: **www.cstl.nist.gov/biotech/strbase/chrom.htm**

[2]The August 1997 report (23,24) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, "1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5´ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used".

[3]Amelogenin is not an STR but displays an 87-base, X-specific band and a 93-base, Y-specific band.

Table 5. The PowerPlex® ESX 17 System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components[3]
D18S51	Fluorescein	286–366	7–10, 10.2, 11–13, 13.2, 14–27
D21S11	Fluorescein	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38
TH01	Fluorescein	152–195	3–9, 9.3, 10–11, 13.3
D3S1358	Fluorescein	103–147	9–20
Amelogenin[4]	Fluorescein	87, 93	X, Y
D16S539	JOE	273–321	4–16
D2S1338	JOE	197–269	10, 12, 14–28
D1S1656	JOE	137–184	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3
D10S1248	JOE	83–127	8–19
FGA	TMR-ET	264–410	14–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2
D8S1179	TMR-ET	203–251	7–19
vWA	TMR-ET	124–180	10–24
D22S1045	TMR-ET	79–118	7–20
SE33	CXR-ET	267–417	4.2, 6.3, 8–20, 20.2, 21, 21.2, 22, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35-37, 39, 42
D19S433	CXR-ET	193–245	5.2, 6.2, 8–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2
D12S391	CXR-ET	130–182	14–17, 17.3, 18, 18.3, 19–27
D2S441	CXR-ET	88–124	8–11, 11.3, 12–17

[1]The length of each allele in the allelic ladder has been confirmed by sequence analyses.

[2]When using an internal lane standard, such as the CC5 Internal Lane Standard 500, the calculated sizes of allelic ladder components may differ from those listed. This occurs because different sequences in allelic ladder and ILS components may cause differences in migration. The dye label and linker also affect migration of alleles.

[3]For a current list of microvariants, see the Variant Allele Report published at the U.S. National Institute of Standards and Technology (NIST) web site at: **www.cstl.nist.gov/div831/strbase/**

[4]Amelogenin is not an STR but displays an 87-base, X-specific band and a 93-base, Y-specific band.

Table 6. The PowerPlex® ESX 17 System Allele Determinations in Commonly Available Standard DNA Templates.

	Standard DNA Templates[1]		
STR Locus	**9947A**	**9948**	**2800M**
D18S51	15, 19	15, 18	16, 18
D21S11	30, 30	29, 30	29, 31.2
TH01	8, 9.3	6, 9.3	6, 9.3
D3S1358	14, 15	15, 17	17, 18
Amelogenin	X, X	X, Y	X, Y
D16S539	11, 12	11, 11	9, 13
D2S1338	19, 23	23, 23	22, 25
D1S1656	18.3, 18.3	14, 17	12, 13
D10S1248	13, 15	12, 15	13, 15
FGA	23, 24	24, 26	20, 23
D8S1179	13, 13	12, 13	14, 15
vWA	17, 18	17, 17	16, 19
D22S1045	11, 14	16, 18	16, 16
SE33	19, 29.2	23.2, 26.2	15, 16
D19S433	14, 15	13, 14	13, 14
D12S391	18, 20	18, 24	18, 23
D2S441	10, 14	11, 12	10, 14

[1]Information about strains 9947A and 9948 is available online at:
http://ccr.coriell.org/Sections/Collections/NIGMS/?SsId=8
Information about the use of 9947A and 9948 DNA as standard DNA templates can be found in reference 25.

9.B. DNA Extraction and Quantitation Methods and Automation Support

Promega offers a wide variety of reagents and automated methods for sample preparation, DNA purification and DNA quantitation prior to STR amplification.

For analysis of database, reference and other single-source samples, we recommend direct amplification from FTA® punches or preprocessing of swabs and nonFTA punches with the SwabSolution™ Kit or PunchSolution™ Kit, respectively. The SwabSolution™ Kit (Cat.# DC8271) contains reagents for rapid DNA preparation from buccal swabs prior to amplification. The procedure lyses cells contained on the swab head and releases into solution sufficient DNA for STR amplification. A small volume of the final swab extract is added to the PowerPlex® reaction. The PunchSolution™ Kit is used to process punches from nonFTA storage cards containing buccal samples prior to direct amplification.

For casework or samples that require DNA purification, we recommend the DNA IQ™ System (Cat.# DC6700), which is a DNA isolation and quantitation system designed specifically for forensic and paternity samples (26). This system uses paramagnetic particles to prepare clean samples for STR analysis easily and efficiently and can be used to extract DNA from stains or liquid samples, such as blood or solutions. The DNA IQ™ Resin eliminates PCR inhibitors and contaminants frequently encountered in casework samples. With DNA-rich samples, the DNA IQ™ System delivers a consistent amount of total DNA. The system has been used to isolate DNA from routine sample types including buccal swabs, stains on FTA® paper and liquid blood. Additionally, DNA has been isolated from casework samples such as tissue, differentially separated sexual assault samples and stains on support materials. The DNA IQ™ System has been tested with the PowerPlex® Systems to ensure a streamlined process. See Section 9.E for ordering information.

For applications requiring human-specific DNA quantification, the Plexor® HY System (Cat.# DC1000) was developed (27). See Section 9.E for ordering information.

For information about automation of Promega chemistries on automated workstations using Identity Automation™ solutions, contact your local Promega Branch Office or Distributor (contact information available at: **www.promega.com/support/worldwide-contacts/**), e-mail: **genetic@promega.com** or visit: **www.promega.com/idautomation/**

9.C. The CC5 Internal Lane Standard 500

The CC5 Internal Lane Standard 500 contains 21 DNA fragments of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases in length (Figure 25). Each fragment is labeled with CC5 dye and can be detected separately (as a fifth color) in the presence of PowerPlex® ESX 17-amplified material. The CC5 ILS 500 is designed for use in each CE injection to increase precision in analyses when using the PowerPlex® ESX 17 System. Protocols to prepare and use this internal lane standard are provided in Section 5.

A low-level artifact peak at approximately 172 bases may be observed with the CC5 ILS 500 in the orange channel. The peak height of this artifact may vary from lot-to-lot and may be labeled by the software. This peak is not used during sizing of the peaks present in the sample.

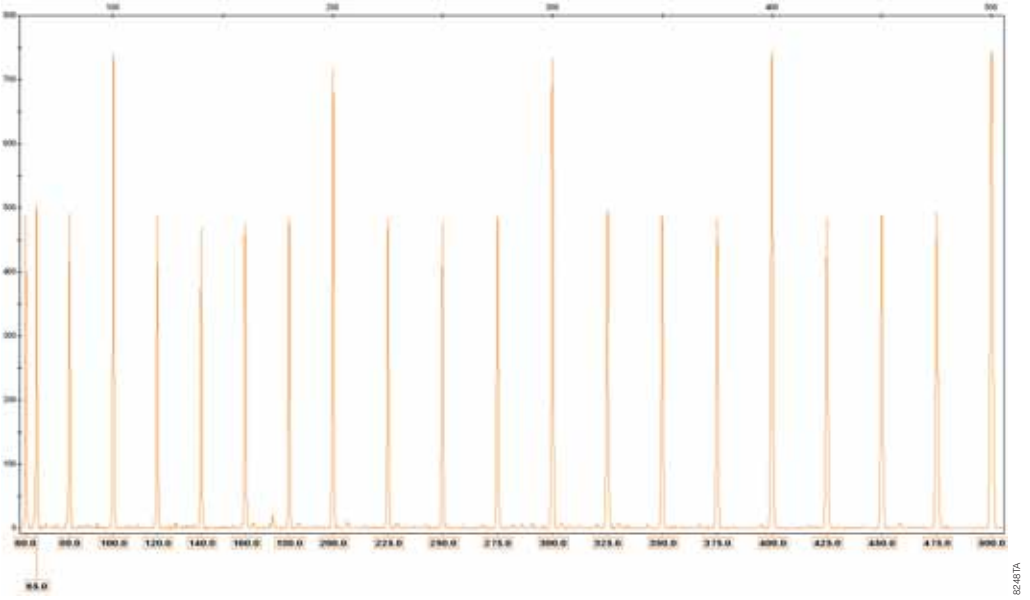

Figure 25. CC5 Internal Lane Standard 500. An electropherogram showing the CC5 Internal Lane Standard 500 fragments.

9.D. Composition of Buffers and Solutions

TE^{-4} buffer (10mM Tris-HCl, 0.1mM EDTA [pH 8.0])

1.21g	Tris base
0.037g	EDTA ($Na_2EDTA \cdot 2H_2O$)

Dissolve Tris base and EDTA in 900ml of deionized water. Adjust to pH 8.0 with HCl. Bring the final volume to 1 liter with deionized water.

TE^{-4} buffer with 20µg/ml glycogen

1.21g	Tris base
0.037g	EDTA ($Na_2EDTA \cdot 2H_2O$)
20µg/ml	glycogen

Dissolve Tris base and EDTA in 900ml of deionized water. Adjust to pH 8.0 with HCl. Add glycogen. Bring the final volume to 1 liter with deionized water.

9.E. Related Products

Fluorescent STR Systems

Product	Size	Cat.#
PowerPlex® ESX 17 Fast System	100 reactions	DC1711
	400 reactions	DC1710
PowerPlex® ESI 17 Fast System	100 reactions	DC1721
	400 reactions	DC1720
PowerPlex® ESX 16 System	100 reactions	DC6711
	400 reactions	DC6710
PowerPlex® ESI 16 System	100 reactions	DC6771
	400 reactions	DC6770
PowerPlex® ESI 17 Pro System	100 reactions	DC7781
	400 reactions	DC7780
PowerPlex® 16 Monoplex System, Penta E (Fluorescein)	100 reactions	DC6591
PowerPlex® 16 Monoplex System, Penta D (JOE)	100 reactions	DC6651
PowerPlex® ES Monoplex System, SE33 (JOE)	100 reactions	DC6751
PowerPlex® Fusion System	200 reactions	DC2402
	800 reactions	DC2408
PowerPlex® Y23 System	50 reactions	DC2305
	200 reactions	DC2320
PowerPlex® 21 System	200 reactions	DC8902
PowerPlex® 18D System	200 reactions	DC1802
	800 reactions	DC1808
PowerPlex® 16 HS System	100 reactions	DC2101
	400 reactions	DC2100
PowerPlex® S5 System	100 reactions	DC6951
	400 reactions	DC6950
PowerPlex® CS7 System	100 reactions	DC6613

Not for Medical Diagnostic Use.

Accessory Components

Product	Size	Cat.#
PowerPlex® 5-Dye Matrix Standards, 310	50µl (each dye)	DG4600
PowerPlex® 5-Dye Matrix Standards, 3100/3130	25µl (each dye)	DG4700
CC5 Internal Lane Standard 500	300µl	DG1521
PunchSolution™ Kit	100 preparations	DC9271
SwabSolution™ Kit	100 preparations	DC8271
2800M Control DNA (10ng/µl)	25µl	DD7101
2800M Control DNA (0.25ng/µl)	500µl	DD7251
Water, Amplification Grade	6,250µl (5 × 1,250µl)	DW0991
5X AmpSolution™ Reagent	500µl	DM1231

Not for Medical Diagnostic Use.

Sample Preparation Systems

Product	Size	Cat.#
DNA IQ™ System	100 reactions	DC6701
	400 reactions	DC6700
Differex™ System*	50 samples	DC6801
	200 samples	DC6800
Tissue and Hair Extraction Kit (for use with DNA IQ™)	100 reactions	DC6740
Maxwell® 16 Forensic Instrument	each	AS3060
DNA IQ™ Reference Sample Kit for Maxwell® 16**	48 preps	AS1040
DNA IQ™ Casework Pro Kit for Maxwell® 16*	48 preps	AS1240
Plexor® HY System*	200 reactions	DC1001
	800 reactions	DC1000
Slicprep™ 96 Device	10 pack	V1391

*Not for Medical Diagnostic Use.
**For Research Use Only. Not for use in diagnostic procedures.

ART® Aerosol-Resistant Tips

Product	Volume	Size (tips/pack)	Cat.#
ART® 10 Ultramicro Pipet Tip	0.5–10µl	960	DY1051
ART® 20E Ultramicro Pipet Tip	0.5–10µl	960	DY1061
ART® 20P Pipet Tip	20µl	960	DY1071
ART® GEL Gel Loading Pipet Tip	100µl	960	DY1081
ART® 100 Pipet Tip	100µl	960	DY1101
ART® 100E Pipet Tip	100µl	960	DY1111
ART® 200 Pipet Tip	200µl	960	DY1121
ART® 1000E Pipet Tip	1,000µl	800	DY1131

9.F. Summary of Changes

The following change was made to the 6/14 revision of this document:

Legal disclaimers were updated.

[a]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175, European Pat. No. 1088060 and other patents pending.

[b]U.S. Pat. Nos. 5,843,660, 6,479,235, 6,221,598 and 7,008,771, Australian Pat. No. 724531, Canadian Pat. Nos. 2,118,048 and 2,251,793, Korean Pat. No. 290332, Singapore Pat. No. 57050, Japanese Pat. Nos. 3602142 and 4034293, Chinese Pat. Nos. ZL99813729.4 and ZL97194967.0, European Pat. No. 0960207 and other patents pending.

[c]STR loci are the subject of U.S. Pat. No. RE 37,984, German Pat. No. DE 38 34 636 C2 and other patents issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, e.V., Germany.

[d]Allele sequences for one or more of the loci vWA, FGA, D8S1179, D21S11 and D18S51 in allelic ladder mixtures is licensed under U.S. Pat. Nos. 7,087,380, 7,645,580, Australia Pat. No. 2003200444 and corresponding patent claims outside the US.

[e]TMR-ET, CXR-ET and CC5 dyes are proprietary.

[f]This product or portions thereof is manufactured and sold under license from GE Healthcare under Australia Pat. No. 692230, Austria Pat. No. E236994, Belgium Pat. No. 0743987, Canada Pat. No. 2231475, EP Pat. Nos. 0743987 and 0851867, France Pat. Nos. 0743987 and 0851867, Germany Pat. Nos. 19581489, 69530286.8 and 0851867, Italy Pat. Nos. 0743987 and 0851867, Japan Pat. No. 3066984, Liechtenstein Pat. Nos. 0743987 and 0851867, Netherlands Pat. Nos. 0743987 and 0851867, Spain Pat. Nos. 2197193 and 2173310, Sweden Pat. Nos. 0743987 and

0851867, Switzerland Pat. Nos. 0743987 and 0851867, United Kingdom Pat. Nos. 0743987 and 0851867, U.S. Pat. Nos. 5,654,419, 5,688,648, 5,869,255, 6,177,247, 5,707,804, 6,028,190, 6,544,744, 7,015,000 and 5,728,528 and other pending and foreign patent applications.

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